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OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

THE FERTILIZATION REACTION IN ECHINARACHNIUS PARMA.

I. Cortical Response of the Egg to Insemination.

E. E. JUST.

Introduction.

So much has already been written about membrane "formation" in sea urchin eggs that one should perhaps hesitate to add to the list of papers on the subject. The observations here reported, however, would seem to fill a gap that has hitherto existed in a critical stage of the fertilization process. We know that the response of the egg to the spermatozoön on insemination first manifests itself in the appearance of a membrane at some distance from the vitellus—by some believed to be already on the uninseminated eggs; by others, to be actually formed *de novo*—but accounts as to why and how this membrane forms differ with different workers even where the same egg has been studied. Thus Herbst, Schücking, Kite and Heilbrunn among others have shown by different methods that the membrane is present on the unfertilized eggs; O. Hertwig originally at least expressed a similar view. R. Hertwig holds this view. On the other hand, Loeb, and the Loeb school generally not only believe that the membrane is formed after insemination but also that its formation is of great significance in the fertilization process; to Robertson, for example, membrane formatïon *is* fertilization. Again, we have the notion of Elder and of McClendon that the "fertilization membrane" is a precipitation membrane formed only in the presence of the intact jelly hull of the egg—a totally erroneous conception as shown by Harvey ('14). (See also, Lillie, '14.) Finally, while it is usually held that the membrane arises simultaneously from all points of the egg, suggestions have not been wanting that the membrane forms as a wave that sweeps over the egg.

Fol first intimated that the membrane lifts from the egg as a wave dwelling on the rapidity with which the process is completed. Unfortunately he used eggs under pressure. Membrane formation as a progressive wave beginning at the site of sperm-entrance has been observed by Wilson who, however, is not at all sure. He says (in a footnote): "I have often observed that the formation of the membrane, in *Toxopneustes*, proceeds like a wave from the entrance-point around the periphery, but this is often irregular." Ries followed the fertilization with the cinematograph; he observed changes which indicated that the membrane forms first at one point.

In 1915 Dr. Tennent informed the writer that he had observed in cases a wavelike membrane formation. According to other workers, however, it would appear that no part of the cortex takes precedence in lifting off the membrane. Thus, Harvey ('10) who worked with *Toxopneustes*, *Hipponoë* and *Arbacia* says: "As observed in the living egg, almost immediately (1½ to 3 minutes) after addition of sperm the membrane substance becomes separated from the egg surface by spaces. These spaces fill with a fluid, unite and enlarge, thus pushing out the membrane some little distance.[1] This statement must refer to the egg of *Toxopneustes* (with which Wilson worked) or to that of *Hipponoë* for Heilbrunn after long experience with the egg of *Arbacia* was never able actually to follow the membrane elevation; he believed that in this egg the membrane lifted simultaneously from all parts of the cortex.

Loeb has followed membrane formation in the egg of *Strongylocentrotus purpuratus* by lowering the temperature of the seawater which retards the process. He gives eight figures of the process described. "The beginning of the process shows itself in a roughening of the hitherto smooth surface of the egg. This is due to the formation of countless tiny vesicles which stand out on the surface of the egg. These small droplets quickly increase in size (through absorption of water) and flow together into larger drops. This goes on until finally the contents of all the drops have run together into a continuous layer around the egg. Hence the surface lamellæ of the tiny droplets form later the fertilization membrane." At higher temperatures, the egg of *Strongylocentrotus* forms a membrane very quickly passing directly from the condition of the uninseminated egg to that with fully formed membrane and wide perivitelline space; thus too in *Arbacia*. (Loeb, *loc. cit.*)

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[1] The reader must not conclude from this statement that Harvey believes that the unfertilized egg possesses a membrane. In this paper he makes a categorical statement that the membrane arises at fertilization. In his 1914 paper he reaffirms this statement.

During a study of the fertilization reaction in the egg of *Echinarachnius* covering several seasons at the Marine Biological Laboratory at Woods Hole, Mass., I have made observations on membrane elevation in this egg where the process though it takes place with great rapidity can nevertheless because of the size of the egg be followed with remarkable ease. I can, therefore, make the unqualified statement that in the egg of *Echinarachnius* membrane elevation proceeds as a wave from the entrance-point of the sperm around the cortex. Moreover, what is more significant, before membrane elevation cortical changes blocking farther sperm entry spread as a wave over the egg uniting finally at the point opposite the entrance-point of the sperm.

OBSERVATIONS.[1]

If eggs of *Echinarachnius* be inseminated with thin sperm suspension they throw off membranes that are fully formed and equidistant from the surface of the vitellus in from two to three minutes. This follows sperm penetration which may take place at any point. Sperm is engulfed by the egg in from fourteen to forty-five seconds after insemination; the membrane begins to lift off in from seven to twenty-two seconds after sperm entry is complete; and membrane elevation is complete in from nine to thirty seconds after it begins. The shortest time recorded from insemination to membrane elevation from all parts of the egg is thirty-nine seconds, all time being taken with a stop-watch of a standard make. In some thirty to sixty seconds after the membrane is off from the egg it fully rounds out, equidistant from the surface of the cytoplasm at all points. The following tables taken from the data give some idea of the time relations. The time is in seconds. The last column ("Membrane Off") gives the time at which the membrane is distinctly off the egg at all points but not yet fully rounded. Each record is from observations of a single egg.

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[1] All observations were made on eggs in a quantity of sea-water; the eggs never suffered compression. Though jelly-free eggs form membranes, most observations were made on eggs with jelly so that the eggs were therefore free from flattening when on the bottom of the dish—the jelly acting as a buffer. Flattening of the eggs might easily lead to erroneous conclusions.

TABLE I.

Insemination.	Sperm Engulfed.	Membrane Begins.	Membrane Off.
0	50	65	80
0	49	64	84
0	30	50	70
0	45	67	
0	30	50	70

TABLE II.

Insemination.	Sperm In.	Membrane Begins.	Membrane Off.
0	30	45	57
0	15	37	53
0		44	66
0		32	57
0		29	39
0		37	48
0		38	47

TABLE III.

Insemination.	Membrane Begins.	Membrane Off.
0	31	42
0	39	46
0	30	50
0	31	44
0	29	43
0	24	41
0	26	42
0	31	49
0	30	45
0	40	51
0	23	49
0	31	50
0	30	45
0	26	44
0	27	52
0	35	45
0	24	46

Average time of appearance of cone 90 seconds.

Average time of fully rounded membrane 134 seconds.

Immediately on insemination, sperm pierce the jelly hull, reaching the vitellus with rapid spiral movements; the moment the tip of the sperm touches the cortex of the vitellus all movements cease, the head and tail in a straight line at right angles to a tangent of the egg surface. Penetration follows as an activity of the egg; the spermatozoön does not bore its way in—the egg pulls it in.[2] After the head has disappeared within the cortex, membrane elevation begins.

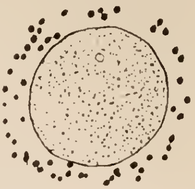
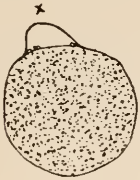


FIG. 1.[1] Freshly shed egg. Its pigmented jelly is as yet unswollen by the sea water.

After the sperm head has completely disappeared within the egg, the cortex reacts to penetration by pushing out a blister at the site of sperm entry (Fig. 2). This blister, which is not optically empty but contains minute drops that wander across this newly formed perivitelline space, may push off at once to a distance equal to the greatest the membrane ever reaches (Fig. 2); or, what is more common, the membrane elevation slowly sweeps (Figs. 3, 4, 5, 6, 7, 8) around the surface of the egg increasing the width of the perivitelline space after having lifted off from the egg at all points (Fig. 9). One may picture the process thus: By escape of substances from the cortex at the



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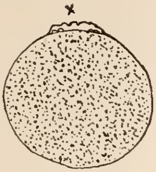

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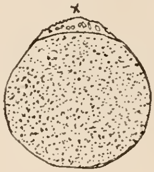

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FIG. 2. An egg ten seconds after the disappearance of the sperm head within the egg.

FIG. 3. Same as Fig. 2.

FIG. 4. Four seconds after the membrane began lifting. Free vesicles beneath the membrane.

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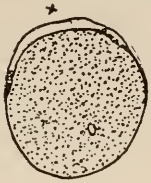
[1] Figs. 1, 9, 10, and 11 were drawn with the aid of a camera lucida. All others are of necessity free-hand sketches. Except in Fig. 1, the jelly hull is omitted. *x* marks the site of sperm entry.

[2] Kupffer and Benecke in 1878 made similar observations on the lamprey egg and reached the conclusion that the sperm is engulfed.

point of sperm entry the membrane thoroughly glued to the egg surface is pushed off; the material continuing throughout the cortex to escape progressively lifts off the membrane around the egg.[1] The droplets are squeezed out of the cortex into the perivitelline space and may reach the membrane as discrete bodies before they fade from view. Often they go into solution more quickly. The membrane thickens slightly after formed.



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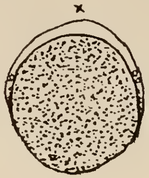

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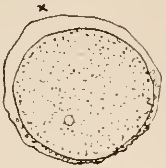
FIG. 5. About two seconds later than Fig. 4.

FIG. 6. Two to three seconds later than Fig. 5. Note vesicles forming.

As the membrane lifts off, it carries away any supernumerary sperm whose activity is in contrast to immobilized sperm previously engulfed by the egg. Not only is it true that at no point at which the membrane has elevated can sperm enter, but also that membrane elevation at a given point though not complete for the whole egg is a bar to sperm entrance at any point on the egg surface. And this is the most significant point in the reaction. *Before the actual elevation of the membrane, some cortical change beginning at the point of sperm entry sweeps over*



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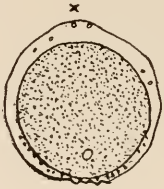

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FIG. 7. About two seconds later than Fig. 6. Membrane incomplete at pole opposite entrance-point of the sperm.

FIG. 8. Twelve seconds after beginning of membrane lifting. The membrane is fully formed but still wrinkled, especially at point at which it lifted last.

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[1] This does not mean, however, that the eggs decrease in size after fertilization.

the egg immunizing it to other sperm; the direct opposite pole of the site of sperm entry is the last point affected.

The site of sperm entry becomes a "point of injury" and is "negative" for any other sperm arriving at this point, all other portions around the egg being positive. This "wave of negativity" moves over the egg, its rate varying with the variations in the time that marks the disappearance of the sperm within the cytoplasm. When only the tip of the sperm head has entered the cytoplasm, the immediate vicinity of the site of penetration alone can not engulf sperm. As more of the sperm head disappears within the cortex of the egg, the "negativity" of the cortex for sperm entry progresses still farther around the egg until at the moment the head has disappeared the egg can engulf sperm only at one point—the pole opposite that at which the sperm entered the egg. A "wave of negativity" thus progressively sweeps over the egg from the point of sperm entry *preceding the actual beginning of membrane lifting.* Before the membrane begins lifting at the site of sperm entry sperm can no longer enter at any point on the egg.[1] From the point of sperm entry a definite gradient of membrane elevation is established, the last point of membrane elevation being at the pole opposite that of successful sperm entry. This gradient, therefore, follows that of diminished susceptibility to sperm penetration initiated at the entrance-place of the sperm.

If several spermatozoa become attached to the vitellus at the same instant membrane elevation starts at each point of attachment no matter what the distance separating them. Thus, two, three or more sperm may be attached at very nearly the same point or at varying distances from each other; in these cases other sperm later in arrival at the periphery of the egg, do not become engulfed, though they may be affixed by the egg. The membrane lifts at these points as blisters; each point behaves with reference to the space between it and an adjacent point of attachment as does the point in monospermic eggs, except that the "waves of negativity" spreading from each move to meet thus diminishing the positive reaction of the cortex between two sperm points. Often, of course, even in heavy insemination a single spermatozoön reaches the egg at some one point ahead of others; in this case the egg is monospermic and so behaves. The observer is indeed struck with the rare occurrence of polyspermy even with heavy insemination.

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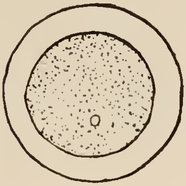
[1] In 1878 O. Hertwig expressed the view that it is the egg plasma itself, if its vitality be unimpaired, which alone can prevent the entrance of more than one sperm.

When a spermatozoön becomes attached not only the penetration of other sperm but also their fixation to the vitellus depends upon the degree of penetration of the attached sperm or perhaps rather on the rate at which the "wave of negativity" is propagated around the egg. Thus, at the beginning of penetration, sperm nearby cannot become attached to the vitellus; those farther removed may become attached, lashing back and forth very vigorously. The heads of those still farther away but not far enough beyond the area of decrement actually to be engulfed are likewise affixed and present a swollen appearance. When the membrane lifts off these affixed sperm are carried with it.



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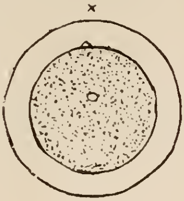

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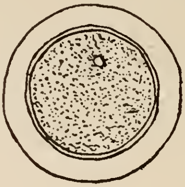

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FIG. 9. Two minutes after insemination. Membrane is fully rounded, equidistant at all points from the now spherical egg.

FIG. 10. About same time as in Fig. 9. Cone is clearly visible.

FIG. 11. Ten minutes after insemination. Egg with hyaline plasma layer formed

The *Echinarachnius* egg, then, would seem to be unusual, since a gradient of susceptibility to the sperm may actually be demonstrated in the cortical changes following sperm entry.

After elevation of the membrane in *Echinarachnius* egg a cone (Fig. 10) of clear cytoplasm forms at the point of sperm entry; sectioned eggs show the sperm within where rotation of the head may begin. This cone[1] disappears; within some ten to fifteen minutes after insemination a thin film forms on the surface of the cytoplasm (Fig. 11) the hyaline plasma layer, or gelatinous film of Loeb.

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[1] The cone and the behavior of sperm that are attached to the cortex but do not enter constitute important checks on the observation of the point of sperm entry.

Experiments are being made at present with the hope of analyzing farther these cortical responses of the *Echinarachnius* egg to the sperm. We may, however, point out in conclusion to these observations that in the progressive membrane lifting and the cortical change that precedes it we have another of the numerous examples of an activity gradient; this of course is distinct from the primary axial (polar) gradient that exists in echinoderm eggs (see Child). In following these waves of cortical change we are actually viewing the propagation of the effect of a stimulus in an irritable cell. One effect of this stimulus of sperm entrance is to alter the plasma to such an extent that it prevents the entrance of other sperm. It is not the membrane that is a block to polyspermy; that block exists before the membrane lifts off. The membrane is merely the sign and consequence of more profound cortical changes.[1] In a way, it is of no significance that the membrane rises sharply into visibility provided these primary cortical changes take place. And indeed I have seen any number of eggs fertilize and develop without throwing off membranes. Such eggs are as incapable of reinsemination as eggs that have formed membranes. In the *Echinarachnius* egg, then, normal development has been already initiated by the sperm when the membrane begins to form.

[1] Says Lillie: "The fundamental mechanism for the prevention of polyspermy is the neutralization of the fertilizin by the anti-fertilizing present in the egg; *i. e.*, the occupancy of the spermophile side-chain of the fertilizin by the anti-fertilizin.

"The question may be raised why such neutralization of the fertilizin is delayed until the moment of fertilization? The answer to this difficulty is fairly clear. The fertilizin is located in the cortex of the egg, and the anti-fertilizin is more deeply situated; they therefore do not interact so long as the cell body as a whole is quiescent. But as soon as the cortical fertilizin becomes activated by union with the sperm it at once begins to attach certain substances in the egg, as demonstrated in the third part of this paper; this sets up diffusion evidenced by escape of pigment, and by cytoplasmic flowing, and the two substances are brought together and interact. While this explanation is partly hypothetical, the spatial separation of the fertilizin and anti-fertilizin and the quiescent character of the cell-body in the unfertilized egg are facts; so also are the movements of diffusion and the cytoplasmic currents set up on fertilization."

LITERATURE CITED.

Child, C. M.

'15 Axial Susceptibility Gradients in the Early Development of the Sea-urchin. BIOL. BULL., Vol. 30.

Elder, J. E.

'13 The Relation of the Zona Pellucida to the Formation of the Fertilization Membrane in the Egg of the Sea-urchin (*Stronglyocentrotus purpuratus*). Arch. f. Entwick., Bd. 35.

Fol, H.

'77 Sur le commencement de l'henogenie chez divers animaux. Arch. Sci. Nat. et Phys. Geneve, LVIII.

Harvey, E. N.

'10 Mechanism of Membrane Formation and Other Early Changes in Developing Sea-urchin's Eggs as Bearing upon the Problem of Artificial Parthenogenesis. Jour. Ex. Zoöl., Vol. 8.

'14 Is the Fertilization Membrane of *Arbacia* Eggs a Precipitation Membrane? BIOL. BULL., Vol. 27.

Heilbrunn L. V.

'15 Studies in Artificial Parthenogenesis. II. Physical Changes in the Egg of *Arbacia*. BIOL. BULL., Vol. 29.

Lillie, F. R.

'14 Studies of Fertilization. VI., The Mechanism of Fertilization in *Arbacia*. Jour. Ex. Zoöl., Vol. 16.

Loeb, J.

'13 Artificial Parthenogenesis and Fertilization. The University of Chicago Press.

McClendon, J. F.

'11 The Relation between the Formation of the Fertilization Membrane and the Initiation of the Development of the Echinoderm Egg. Science, N. S., 33, No. 845.

Ries, Julius.

'09 Kinematographic der Befruchtung und Zellteilung. Arch. Mik. Anat., Bd. 74.

Robertson, T. B.

'12 Studies in the Fertilization of the Eggs of a Sea-urchin (*Strongylocentrotus purpuratus*) by Blood Sera, Sperm, Sperm Extract, and other Fertilizing Agents. Arch. Entwick., Bd. 35.

Wilson, E. B.

'00 The Cell in Development and Inheritance. New York, The Macmillan Co.

THE FERTILIZATION REACTION IN ECHINARACHNIUS PARMA.

II. The Role of Fertilizin in Straight and Cross Fertilization.

E. E. JUST.

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I. Introduction.

The behavior of the sperm cells with reference to egg-extractives and the significance of this for the mechanism of fertilization has been analyzed in detail only in *Arbacia* and in *Nereis* (Lillie, '13, '14). It is true that the agglutination reaction had been previously studied by various workers, but the rôle of this phenomenon in fertilization as a specific reaction was not suggested; and it is on this specificity that the fertilizin theory of Lillie in large part rests; that is, that the egg alone secretes the sperm-agglutinating substance and that this substance is necessary for fertilization. It seemed desirable by study of this agglutinating reaction to determine its rôle in fertilization in some other form; this has been done in *Echinarachnius parma*. The present paper is a contribution to the analysis of the rôle of the sperm-agglutinating substance as revealed by a study of its behavior in straight and in cross fertilization. The results conform with those of Lillie, the data on cross fertilization seemingly at variance actually giving support to the view that the agglutination reaction is specific. The sperm agglutinin of *Echinarachnius* is, therefore, identical in behavior with the fertilizin of *Arbacia*.

Although observations on *Echinarachnius* were made during 1914, and 1915, all experiments here reported except those cited on page 22 are from those made, at the Marine Biological Laboratory at Woods Hole, Mass., during the season of 1917, beginning early in June. The experiments fall into two groups: those on iso-agglutination and straight fertilization in *Echinarachnius* (Part II.) and those on hetero-agglutination and cross fertilization with *Arbacia* (Part III.).

II. Sperm Agglutination and its Significance in Echinarachnius.

The mere fact that sperm are agglutinated by substances dissolved in sea-water is of no special significance for us since many substances will accomplish this; among others, alkalis, polyvalent salts (Gray), and snake venom (Noguchi); but the agglutinations by these agents are distinctly toxic and we must, therefore, at the outset exclude them from consideration. They must in no wise be confused with agglutination produced by egg secretions. What is significant is that the agglutinin produced by eggs behaves in a characteristic fashion, may be quantitatively studied, and is a necessary part of the fertilization reaction.

A. *Sperm Agglutinin Production in Echinarachnius.*

Eggs of *Echinarachnius* charge sea-water with a substance that agglutinates *Echinarachnius* sperm. If a drop of this charged sea-water be added to a sperm suspension the sperm rapidly form clumps. The eggs give off this agglutinating substance in the absence of the jelly hulls enclosing them. Stale eggs give off relatively little and immature eggs none, of the agglutinin.

1. *Quantitative Method for Determining Agglutinin Production by Ova.*

By the method of successive dilutions of the sea-water taken from above *Echinarachnius* eggs after they have settled one may obtain quantitative estimates of the agglutinating power of the egg-secretion. This method, used by Lillie on *Arbacia*, briefly is as follows: A series of successive dilutions of the sea-water above *Echinarachnius* eggs is made, each dilution being one half the preceding. Usually I started with a 1/100 dilution of the agglutinin charged sea-water; a half dilution gives 1/200. Thus the series 1/100, 1/200, 1/400, 1/800, etc., is made. As noted below, series of 1/2, 1/4, 1/8, 1/16, etc., were likewise employed, so too, 1/10, 1/20, 1/40, etc. The agglutinating power of these dilutions is tested on sperm suspensions until the reaction comes negative. The last dilution to give a positive reaction rates the power of the agglutinin.

To observe the agglutination, drops of sperm suspension are mounted on a slide the cover slip of which is supported by glass rods, the slide being placed on the stage of the microscope in focus under low power. Into this sperm suspension, by means of a capillary pipette attached to a rubber tube, is blown a drop of the sea-water from the eggs. This microscopic method, as will be shown beyond, is indispensable for a comparative study of the agglutination reaction, or indeed for accurate quantitative determinations.

Agglutination may also be observed by adding two or three drops of milky sperm suspension to two cubic centimeters of strong egg-water.[1] The sperm form clumps of such size that they are clearly visible to the naked eye, the sea-water between the masses remaining clear, so sharp is the reaction. This method suffices merely to indicate the *fact* of agglutination, and this only in high concentration. It was employed but seldom in these experiments as will be noted in the following pages. Indeed, by this method alone the critical points in this whole work would scarcely have been determined.

The methods for handling eggs and sperm are important since the state of the egg or sperm suspension conditions the experiment. To obtain eggs relatively free of debris, a mass of ovaries in two or three times their bulk of sea-water is strained through cheese cloth. Or, the animals may be rapidly opened and placed aboral side down in dry watch glasses and dry eggs collected as they exude from the genital pores. Also, eggs may be got as they are normally shed by animals in separate glasses. Sperm is collected from those males that have been opened, dried and placed aboral side down in dry watch glasses, and that soon shed sperm being pipetted off as needed. The sperm suspensions mentioned below, unless specifically noted to the contrary, were always one per cent. suspensions made by the addition of one drop of dry sperm to ninety-nine drops of sea-water. It is imperative to make up the suspension only as needed, for sperm reactions show wide variations depending upon freshness and upon concentration which likewise is a factor in ageing; the less concentrated suspensions deteriorate faster than more concentrated. Finally. everything was kept scrupulously clean, and all means taken to prevent contamination which might easily have invalidated whole series of experiments.

(*a*) *Variations in Fertilizin Production.*—Eggs vary in their capacity for producing fertilizin. In part this variation may be due to the fact that some suspensions do not consist wholly of eggs but may contain bits of tissue, etc.; such a suspension will be inferior to another of equal concentration but containing eggs only. However, this does not fully explain the variation, for it is a simple matter by exercising care to procure, as mentioned above, from ovaries in sea-water clean egg suspensions entirely free from bits of tissue; these eggs as a class (which to avoid circumlocution I designate "ovary eggs") show wide variations and are inferior to dry or shed eggs, *i. e.*, those deposited by animals in clean dry dishes. Except for eggs mentioned on page (21) as normally resistant to fertilization, dry eggs produce more agglutinin than "ovary eggs" but show less variation.

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[1] By egg water is meant sea-water taken from above eggs that have stood for a time.

The following observations from a large body of recorded data may now be cited.

1. July 11, 9:30 A.M.: To 1/2 c.c. of dry eggs sea-water was added to the 10-c.c. mark. At 10:10 7 c.c. of the supernatant sea-water was withdrawn and filtered. Tested with freshly prepared 1 per cent. *Echinarachnius* sperm, this withdrawn agglutinin charged sea-water was negative at 1/3,200 dilution and gave an 8 second reaction at 1/1,600.

2. July 11, 9:40 A.M.: Ovaries of 8 females in 20 c.c. of sea-water strained. Sea-water added to 100 c.c. Eggs settled at the 7 c.c. mark. At 10:20, 90 c.c. of supernatant sea-water decanted and filtered, which when tested on sperm came negative at 1/400 and gave a 6 second reaction at 1/200.

3. July 10, 10:30 A.M.: To 1.5 c.c. of dry eggs 8.5 c.c. of sea-water were added. 11:00 A.M. 8 c.c. of supernatant sea-water decanted and filtered, 8 c.c. being added to the eggs. 11:00 A.M. to 12:10 P.M., supernatant water of the *second washing* gave a 6-second reaction at 1/12,800 dilution.

Comparison of (1) and (2) shows that a 5 per cent. suspension of dry eggs yielded fertilizin of 1/1,600 power while a 7 per cent. suspension of ovary eggs contained fertilizin of but 1/200 power. Also, (3) shows us that after a *second washing* a 15 per cent. dry egg suspension gave fertilizin rated at 1/12,800 power. Without exception, the most powerful fertilizin suspensions were from shed eggs. These results are typical of a large number of observations.

(*b*) *Fertilizin Production by Washed Eggs.*—Lillie has shown that in *Arbacia* it is practically impossible by washing to remove all the fertilizin; as long as the egg lives it produces the agglutinating substance. The egg of *Echinarachnius* is not so hardy as that of *Arbacia;* becoming physiologically inert after remaining in sea-water for a time, it ceases to give off fertilizin though far from dead. This cessation is gradual, with each successive washing the fertilizin produced being less. Two experiments are cited.

1. July 2, 10:00 A.M.: Eggs from 4 females washed 5 times in 250 c.c. of sea-water at each washing. 2 c.c. of eggs after the fifth washing. At 12:00 M. 210 c.c. of supernatant water withdrawn and filtered, 210 c.c. of sea-water being added to the eggs. A sample of this removed at 1:30 P.M. on testing gave at 1/1,600 dilution a 10 second reaction. This is equal to morning tests. Washed 5 times during the afternoon by removing 210, 217, 203, 190, and 205 c.c. of water and adding in each case the quantity withdrawn. Tested at 8:30 P.M., supernatant sea-water gave an 8-second reaction at 1/1,600. July 3, 9:00 A.M. 210 c.c. of supernatant sea-water removed, 210 c.c. added. Tested during the day to 1:30 P.M. negative.

2. July 3, 9:10: Ovaries of 5 females in sea-water strained. Sea-water added to 100-c.c. mark. Washings (75 c.c. of water removed each time) as follows: 9:30, 9:34, 9:38, 10:05, 10:12 during the morning and at 1:50, 2:30 and 2:38 during the afternoon. Tested at 9:40, 10:50 and 1:30 gave 10 second reactions at 1/100. At 2:40, 8-second reaction on 1/50 dilution. 2:55 P.M. 75 c.c. of supernatant water removed, 75 c.c. added. 5 P.M., reaction faintly positive at 1/50. July 4, 10:30 A.M. 75 c.c. removed, 75 c.c. added. 11:10 A.M. negative when tested.

2. *Source of Fertilizin.*

In *Arbacia* only mature eggs produce fertilizin; immature eggs are incapable of secreting the agglutinating substance; other tissues do not elaborate it, nor does it occur in the perivisceral fluid (Lillie, '13, '14). In *Echinarachnius* mature eggs alone with or without jelly yield fertilizin to the sea-water. Neither the immature egg nor the body-fluid is capable of charging sea-water with sperm agglutinin.

(*a*) *Comparison of Ripe Eggs With and Without Jelly.*—Though the jelly is loaded with fertilizin, it does not form the fertilizin for jelly-free eggs are capable of charging the sea-water with the sperm agglutinating substance. The jelly hulls enclosing *Echinarachnius* eggs may be removed by shaking or by treatment with acid. The method of removing the jelly by shaking demands care since agitation may cause injury to the eggs. If shaking brings about the binding of fertilizin we should get a cessation of fertilizin production. This should not be attributed to mere loss of jelly, though it might be so interpreted by some. Again, it might be argued that the use of acid to remove the jelly might injure the egg; this objection could be raised only in the event that fertilizin is no longer produced by the egg after acid treatment, which is at least remotely possible. Certainly, it would be gratuitous to argue that if the fertilizin continues to be produced after acid treatment the acid carries the fertilizin from jelly to egg! In short, after shaking or after acid treatment the cessation of fertilizin production would not prove that the jelly forms fertilizin. However, after both acid treatment and gentle shaking the eggs still produce fertilizin.

Echinarachnius eggs may be shed without jelly. Often in a given lot of eggs some will be found without jelly, and on three occasions during the summer I observed eggs shed *not one of which had a jelly covering.* These eggs proved to be normal in every respect; secreted fertilizin, were capable of fertilization, and of normal development.

I cite three experiments:

1. One c.c. of dry eggs was placed in 50 c.c. of sea-water plus 3 c.c. of $n/10$ HCl. Gently stirred to prevent clumping and washed in four changes of sea-water. Left in 50 c.c. of sea-water. Examination revealed absence of jelly in most eggs. Tested after one hour, the egg water gave an 8-second reaction at 1/200 dilution.

2. One c.c. of eggs was gently shaken five or six times in 10 c.c. of sea-water. Most of the eggs lost their jelly. The eggs were washed 5 times in 10 c.c. of sea-water and left finally in the cylinder in 10 c.c. of clean sea-water. On settling the eggs were at the 0.5-c.c. mark; tested, the supernatant sea-water gave at 1/400 dilution a 6-second reaction.

3. One c.c. of dry eggs shed without jelly was placed in 24 c.c. of sea-water. Tested an hour later the egg-water gave at 1/1,600 dilution a 6 second reaction. After a sixth washing in 3 hours these eggs produced agglutinin giving an 8-second reaction at 1/800 dilution.

Whatever may be the interpretation of the experiments with eggs freed of jelly through shaking or acid treatment, the conclusion drawn from the results with these eggs lacking jelly when shed is inescapable: in *Echinarachnius* as in *Arbacia* the fertilizin is secreted by the egg, however much the jelly may be loaded with it.

(*b*) *Study of Immature Eggs.*—During the season one may always obtain immature sand-dollars. Indeed one gets the impression that there is a rhythm in the breeding season. Loeb's observations ('15) would suggest that this is true of the California urchin, while Tennent[1] has noted the phenomenon which suggests lunar periodicity in the breeding of the Atlantic urchins. I was able, therefore, early in the season to determine quite definitely that immature *Echinarachnius* eggs do not give off fertilizin. Thus, on July 3, immature eggs from three females were in turn tested. The supernatant sea-water from none during ten hours gave an agglutination reaction. Ovarian tissue, likewise, procured after removing the eggs by straining, does not produce a sperm agglutinin. The fully matured egg alone with or without jelly elaborates fertilizin.

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[1] Dr. Tennent also tells me that in Greece the gonads of urchins used as food appear on the market only during certain moon phases; at other times the animals are spent.

(*c*) *Study of Perivisceral Fluid.*—Finally, repeated trials with with perivisceral fluid from mature or immature females never gave agglutination. Thus, at 2:40 P.M., July 3, drops of 1 per cent. *Echinarachnius* sperm suspension placed on slides when tested with perivisceral fluid from three females showed no agglutination. Each of the three samples of fluid was then diluted with clean sea-water 1/2, 1/4, 1/8, 1/16. The reaction with fresh sperm suspension in all cases was negative.

To sum up, we may say that mature *Echinarachnius* eggs in sea-water give off a substance that agglutinates *Echinarachnius* sperm; this substance the eggs will form in the absence of their jelly hulls. Immature eggs do not secrete the agglutinin nor does the ovarian tissue or perivisceral fluid.

The agglutinin of *Echinarachnius* is more powerful than that of *Arbacia*, as can be readily gleaned from a study of Lillie's work. An equal quantity of *Echinarachnius* eggs will give off more fertilizin in a given length of time than *Arbacia* eggs, but *Echinarachnius* eggs will cease production before *Arbacia* eggs. This is doubtless correlated with the greater irritability of *Echinarachnius* egg as evidenced both by its susceptibility to mechanical agitation and its earlier loss of fertilizing power.

It can be readily shown that this loss of fertilizing power is gradual and farther that it runs parallel with lessening fertilizin production.

B. *Fertilizin Production as an Index of Fertilization Capacity.*

While it is true that only mature eggs are capable of producing the agglutinin, this capacity varies between wide limits. One cannot foretell, therefore, the agglutinin production of a given lot of eggs in a given quantity of sea-water. Often eggs which on microscopic examination are found to be fully matured yield no agglutinating substance. Since, as shown above, immature eggs do not secrete fertilizin nor does the jelly form it, the secretion must occur after the formation of the polar bodies. Fertilizin production, therefore, may be regarded as the sign of *physiological maturity.* This is borne out by a study of the parallel between fertilization capacity and fertilizin production. Eggs incapable of fertilization either have produced no fertilizin, unripe eggs, or have lost fertilizin, stale (washed) eggs.

1. *Ovary Eggs and Dry Eggs.*

As has been mentioned above, by cutting up ovaries in sea-water and straining the mass one may procure fairly clean egg suspensions free from bits of tissue. These are the "*ovary eggs.*" Dry or shed eggs are those deposited by the animals in clean dry dishes.

"*Ovary Eggs.*"—Different lots of "ovary eggs" vary greatly with respect to the agglutinin production, as repeated observations showed. A ten per cent. suspension made up of eggs from one female may give a positive reaction on 1/6,400 dilution while another ten per cent. suspension from another female may show little or even no agglutinative power. The capacity for fertilization corresponds so that following insemination ninety per cent. or more of the eggs with strong agglutination action cleave; very few of the eggs of no agglutinative action cleave. This is due to the fact that by cutting up the ovaries one obtains eggs of different degrees of ripeness. One lot from a given female may consist of fully ripened eggs while another lot contain scarcely any ripe eggs despite their appearance. If after washing the same pieces of ovaries several times, one collects eggs after each washing, the percentage of fertilization drops with each washing; the first lot of eggs is made up doubtless of those ready to be shed and so are fully ripened; successive lots are less mature. Moreover, dishes of "ovary eggs" usually contain some ovocytes. Without exception among these eggs those that fail to develop, give off no agglutinin; but those that have great capacity for fertilization show high agglutinating power.

"*Dry Eggs.*"—Dry or shed eggs are perfectly ripe, of high agglutination action, giving practically one hundred per cent. cleavage, beautiful large gastrulæ, and vigorous hardy plutei. The difference between these and "ovary eggs" is striking. Apparently when eggs are shed they are fully ready for fertilization. Some years ago I observed similar behavior in *Asterias.* Eggs cut from ovaries may or may not fertilize: if they do cleave, subsequent development may be uncertain. Eggs naturally shed on the other hand, *may be inseminated at once*, fertilize easily, and develop perfectly, giving rise to larger and more vigorous larvæ. It would be a mistake to reach conclusions concerning the behavior of eggs and sperm unless these products have also been studied as they are normally shed. And I am disposed to believe that a great deal of the variability of eggs, so called physiological condition met with by workers, is due to the use of eggs not perfectly ripe. Dry eggs give powerful agglutinative suspensions.

2. *Washed Eggs.*

It is generally known that eggs lose their capacity for fertilization through remaining in sea-water, though the resistance to sea-water may vary within very wide limits depending upon the species of egg. Thus, the *Platynereis* egg can no longer be fertilized after six to ten seconds in sea-water, whereas the *Arbacia* egg may still be fertilized after several hours. In *Echinarachnius* the power to withstand sea-water varies with the kind of egg ("ovary egg" or dry), the time of season, the temperature, amount of mechanical shock, amount of sea-water used, etc. The most important factor is the condition of the egg since the most perfectly ripened eggs, *i. e.*, shed eggs, lose fertilization capacity earliest. There is an optimum period for fertilization and this gradually passes off as the eggs lie in sea-water. Eggs may lose their fertilization capacity in from two to fifteen hours; washing the eggs repeatedly will hasten this loss. Thus,

(*a*) 1 c.c. of eggs, samples of which when tested were fertilizable, was suspended in 99 c.c. of sea-water at 4:30 P.M., July 6. The supernatant sea-water gave on 1/800 dilution an 8-second reaction. Next day at 9 A.M. these eggs were unfertilizable before or after washing in 100 c.c. of sea-water. The superantant sea-water was negative to *Echinarachnius* sperm when tested throughout the day.

(*b*) The following is typical of a large number of experiments. On July 6 at 8 A.M., 2 c.c. of eggs were collected from three fine females washed in 5 c.c. of sea-water and strained. They were washed seven times, agglutination tests and inseminations being made at each washing. The table gives the data.

EGGS FROM OVARIES OF THREE FEMALES.

Time.	Washings. No.	Agglutination Reaction.			Per Cent. Cleavage.
		C.c. of Sea-water Used.	Time in Seconds.	Dilution.	
8:30	1	100	8	1/1600	90
9:00	2	200	8	1/100	90
9:15	3	100	6	1/800	85
9:40	4	200	6	1/400	80
10:00	5	200	6	1/200	70
12:00	6	75	4	1/50	10
12:10	7	85	4		10

Began with 4 c.c. of eggs, ended with 2 c.c.

Two points are worthy of note respecting this table: First, the amount of water used in washing was unfortunately not the same throughout; and second, which is unavoidable, the quantity of eggs diminished with each washing. These two factors suffice to account for apparent irregularities in per cent. of cleavage and in agglutination. Thus, nos. 1 and 2 give the same per cent. of cleavage, whereas in no. 2 the agglutination reaction is but half as strong, but it is also true that twice as much sea-water was used in no. 2. The time between the washings is likewise irregular; this presumably would affect the amount of agglutinin present in the sea-water. However, the end result is unmistakable: low fertilization and low agglutination reaction are parallel. Since freshly made sperm suspensions were used for each insemination, the only other possible explanation is that the eggs die in large numbers. This is rather hazardous if not gratuitous since the eggs may make abortive attempts at development.

3. *Eggs Normally Resistant to Fertilization.*

On July 15 I procured 7 c.c. of dry eggs which were placed in 65 c.c. of sea-water. Also, by placing ovaries in sea-water for a few minutes before straining I obtained 3 c.c. of eggs which seemed to be unusually fine. Eggs from both lots were inseminated for preservation of a normal series. To my chagrin relatively few of these eggs segmented. July 16 gave similar results. Not until later did experiment reveal the cause of this failure to segment. The observation of July 20 may serve as a typical case.

At 10:10 A.M., dry *Echinarachnius* eggs were placed in sea-water; total bulk of eggs plus sea-water equalling 10 c.c. (*Lot A*.)

10:20 A.M., 10 c.c. of ovaries plus 90 c.c. of sea-water were placed in a cylinder which was gently inverted three times. (*Lot·B.*)

10:50 A.M., 7 c.c. of sea-water removed from eggs of *Lot A* filtered and diluted as follows: 1/10, 1/20, 1/40, 1/80, 1/160. 7 c.c. of fresh sea-water added to eggs. Trials with sperm of two males gave a 6-second reaction at 1/20.

11:20 A.M., 6 c.c. of supernatant sea-water removed and filtered, dilutions being made as before. (6 c.c. of fresh sea-water added to eggs.) 4-(?)-second reaction at 1/10 dilution; very faintly positive.

11:50 A.M., 5 c.c. of supernatant sea-water removed and filtered with dilutions as before, 6-second reaction at 1/10. Eggs inseminated 3:00. These normally fertilized eggs show very few cleavages. *Lot B* gave comparable results.

If this were a long series of washings extending over two or three days, one might suggest that these eggs no longer fertilize or give the agglutination test because they are dead. One could scarcely have the hardihood, though, to venture that the eggs die after remaining in sea-water some ninety minutes. Rather these eggs fail to fertilize, as was discovered during the next two days because of some membrane condition, for on the addition of ether to the sea-water, eggs, of which but 1 per cent. showed cleavage in normal sea-water, gave 90 per cent. cleavage and normal larvæ. I might designate these eggs as over-ripe following R. S. Lillie's usage since they behave so much like over-ripe *Asterias* eggs. The cleavage percentage of these eggs is always low and their agglutinin production feeble. Unfortunately, I failed to study the agglutinative power of these ether-treated eggs.

4. *Fertilizin Production After Membrane Formation.*

Observations on eggs with membranes—which were not made before the season of 1918—show that after insemination or successful butyric acid treatment fertilizin production ceases.

(*a*) *Eggs with Fertilization Membranes.*—Lillie found ('14, pp. 553–557) that in *Arbacia* fertilized eggs with jelly removed by shaking ceased to give the fertilizin test after five washings (extending over a period of one hour and forty-three minutes), the last washing being made at two hours and twenty-three minutes after insemination; while an equal quantity of eggs of the same inseminated lot *with* jelly still gave a test of over seventy-five seconds. When these latter came negative is not stated. From this and duplicate experiments Lillie concluded that after fertilization the egg no longer produces fertilizin; the positive tests with jelly-covered eggs show merely that the jelly is saturated with the secretion. I have repeated this experiment with *Echinarachnius* eggs and have also made some others slightly different. My findings agree with those of Lillie.

An experiment of August 1 is typical of a number:

9:15 A.M. Two tubes (*A* and *B*) each with 0.5 c.c. of eggs from the same female plus 9.5 c.c. of sea-water; 9:16 A.M. inseminated; 9:18 A.M. all eggs have membranes; 9:20 A.M. tube *B* gently shaken to remove jelly; 9:23 A.M. jelly off every single egg, membranes intact. By means of a capillary pipette attached to a rubber tube supernatant sea-water carefully removed from each tube as follows: 9:35, 9:45, 10:00, 10:16, 10:31, 11:00 and 11:13 A.M.; in each case 8 c.c. being removed and 8 c.c. of fresh sea water added. At 11:05 A.M. at 1/1 dilution *B* is slightly positive (?); at 11:15 A.M. and thereafter *B* is negative at 1/1 dilution. *A* at 1/8 dilution gave an 8-second reaction. 99 + per cent. of the eggs in both lots developed normally.

Thus in two hours after insemination (seven washings) the tests on jelly-free eggs came absolutely negative. (*Vide supra*, Lillie's results.)

The data on some of these experiments seemed to indicate that the fertilizin is not lost in any given time but rather after a certain amount of washing. If this be true, a certain amount of water ought to remove from a given lot of eggs the free fertilizin practically at once. The following experiments show this:

Aug. 2, 9:40 A.M. Two cylinders (*A* and *B*) each with 0.5 c.c. of eggs from the same female plus 9.5 c.c. of sea water. Both inseminated. 9:43 A.M. *B* shaken. Eggs have 100 per cent. membranes in both *A* and *B;* none have jelly in *B*.

9:50 A.M. Lots *A* and *B* each in 250 c.c. of sea-water.

9:53 A.M. *A* is positive at 1/2 dilution; *B* is negative at 1/1 dilution, never again positive.

Aug. 3, 9:58 A.M. 2.5 c.c. of eggs from one female plus 7.5 c.c. of sea-water inseminated and divided into two equal lots—*A* and *B*. *B* shaken. *A* and *B* each added to 250 c.c. of sea-water. 10:05 A.M. *B* positive at 1/1 dilution. 10:07 A.M. 190 c.c. of sea-water removed from *A*, 200 c.c. of sea-water removed from *B*. 190 c.c. and 200 c.c. of sea-water added to *A* and *B* respectively. 10:25 A.M. *A* barely positive at 1/8 dilution; *B* is negative at 1/1.

These observations bear out findings of June: while making experiments with shaken eggs I tested fertilized jelly-free and membrane-free eggs and never obtained a positive test. Evidently, Lillie's assumption "that all free fertilizin is fixed *at the moment of* fertilization or membrane formation" is correct.

(*b*) *Eggs with Butyric Acid Membranes.*—If eggs be successfully treated with butyric acid (optimum exposure to optimum concentration of butyric acid in sea-water) they form membranes. In highly successful cases one may obtain one hundred per cent. membranes. These eggs behave as do eggs with fertilization membranes. If washed by repeated removal of the supernatant sea-water they cease producing fertilizin within two hours after five or six washings. If eggs be washed by removal directly to a large quantity of sea-water the residual fertilizin (in the jelly which may not be removed by butyric acid) is washed away so that when tested these eggs are negative; while an equal bulk of unfertilized eggs *without* jelly suspended in a like amount of sea-water will still give the reaction.

We may conclude that after membrane formation induced by sperm or by butyric acid eggs no longer secrete fertilizin.

According to Lillie's studies, and likewise Moore's account ('16), fertilization capacity and fertilizin production in *Arbacia* run parallel. I have found the same to be true in *Platynereis* and *Nereis*. Farther, *Nereis* exhibits a very striking phenomenon for even slight washing renders impossible initiation of development by warming. In the eggs of *Echinarachnius* the measure of fertilizability is agglutinin production.

C. *Summary of Part II.*

We have shown (1) that *Echinarachnius* eggs in sea-water liberate a substance that agglutinates *Echinarachnius* sperm. This substance, fertilizin, which can be quantitatively studied is formed most abundantly in fully ripened (shed) eggs. The substance is lost by eggs repeatedly washed. It is formed by ripe eggs with or without jelly but is not formed by immature eggs nor is it found in perivisceral fluid. (2). Fertilizin production is an index of fertilization capacity. Eggs highest in fertilizin content, shed eggs, fertilize in largest numbers. Washing the eggs decreases both the fertilizin secretion and the percentage of fertilization. Eggs normally resistant to fertilization yield little fertilizin. After membrane formation fertilizin production ceases.

III. CROSS FERTILIZATION AND AGGLUTINATION IN *Echinarachnius* AND *Arbacia*.

We have seen in Part II. that the eggs of *Echinarachnius* in sea-water liberate a substance comparable in every way to the substance, fertilizin, liberated by *Arbacia* eggs. I have, therefore, used the term fertilizin for the *Echinarachnius* substance. The problem that next presented itself was the study of cross-fertilization in these two echinids whose sperm behave in the same way to their egg secretions. In a relatively short time, a rather curious, but as it turned out merely apparent, contradiction was in evidence: The cross was highly successful in one direction only and the agglutination reaction ran counter to and not parallel with cross fertilization capacity. This seemed indeed worthy of study. A great deal of time, therefore, was devoted to repeating the initial attempts at cross fertilization and to determining the nature of, and the part played by, the hetero-agglutinating substance.[1]

In this section I shall present first the results of the cross fertilization and second some experiments which attempt to analyze the significance of the hetero-agglutination.

A. *Reciprocal Cross Fertilization of Echinarachnius and Arbacia.*

With various methods many echinid crosses have been obtained by a large number of workers; of these, the crosses by Tennent and by Vernon may be particularly mentioned because of the widely differing forms employed. The value of these methods is discussed by Tennent and need not be commented on here. Moreover, my object was not so much to test the relative value of these methods for *Arbacia-Echinarachnius* crosses as to test under the same conditions the response of the *Arbacia* egg and of the *Echinarachnius* egg to foreign sperm. Comparison of the capacity of the eggs for cross fertilization is made only where the same method was employed on both eggs. And I may say at once that while it is relatively easy to fertilize *Echinarachnius* eggs with *Arbacia* sperm, the reciprocal fertilization is extremely difficult.

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[1] Abundant material of both straight and cross fertilizations was fixed in a number of fluids. The results of the study of this will appear later.

Three methods were used to obtain cross fertilization: allowing the eggs to stand, treating them with alkali, and subjecting them to concentrated sperm. In each experiment reciprocal crosses[3] were made, but it will be convenient to consider them separately. I shall begin with the *Arbacia* ♀ × *Echinarachnius* ♂.

1. *Arbacia* ♀ × *Echinarachnius* ♂.

1. *Arbacia eggs allowed to stand* in sea-water upward to thirty-six hours show little development following insemination with *Echinarachnius* sperm. For example, on July 2 I made an observation subsequently repeated throughout the season:

July 2. Eggs of *Arbacia* placed in a finger bowl of sea-water from which two samples were removed at hour intervals, from 8 A.M. to 10 P.M.; one sample inseminated with *Echinarachnius* sperm, the other uninseminated control. Hourly inseminations were continued during the next day until 4:00 P.M., a final insemination at 6:00 P.M. One lot, 24-hour, showed 1 per cent. cleavage, its control about 0.1 per cent.

Results with shed eggs placed in sea-water without subsequent washing and with eggs thoroughly washed by changing the water repeatedly show slight differences.

2. *With alkali treatment* the results are practically negative for the amounts of alkali used; whenever development followed the per cent. was extremely low.

July 7, 5:00 P.M. Eggs in 10 c.c. of sea-water plus 2 drops of *n*/10 KOH were inseminated with *Echinarachnius* sperm. 6:40 P.M. Not a single egg formed a membrane. Later none of these developed.

3. *Treatment with concentrated sperm* gave the best results between one and three per cent. of eggs developing. For example, I quote from a long experiment of July 9.

5:00 PM. Five drops of dry eggs from one *Arbacia* were put in each of four watch glasses *A*, *B*, *C*, *D*. To *A*, one drop of *Echinarachnius* sperm suspension added and 5 c.c. of sea-water; to *B*, one drop of *Echinarachnius* sperm suspension and after five minutes 5 c.c. of sea-water; to *C Arbacia* sperm and 5 c.c. of sea-water; *D*, uninseminated control. *A* gave 1 per cent. cleavage; *B* 2 per cent.; *C*, 95 per cent.; and *D* not *one membrane or a cleavage.*

Washing the eggs thoroughly would seem to be beneficial to treatment with concentrated sperm but the data on this I deem at present insufficient to be conclusive.

On the whole, the cross, *Arbacia* ♀ × *Echinarachnius* ♂ *is* not highly successful; often none of the methods gave results. The reciprocal is not so difficult.

2. *Echinarachnius* ♀ × *Arbacia* ♂.

The cross *Echinarachnius* ♀ × *Arbacia* ♂ as well as the reciprocal cross was made usually during agglutination experiments. Thus, after removing the supernatant sea-water from eggs for agglutination tests these could be used for straight or cross fertilization. This method, in addition to being economical, made possible the performing of a large number of experiments during the time the *Echinarachnius* eggs are at their best.

1. *Allowing the Eggs to Stand.*—Although as mentioned above, *Echinarachnius* eggs are not so resistant to sea-water as *Arbacia* eggs, after three to four hours in sea-water they develop following insemination with *Arbacia* sperm. Thus on July 4, *Echinarachnius* eggs were inseminated with *Arbacia* sperm at ten-minute intervals up to three hours. The best lot of eggs was that inseminated after two hours in sea-water and showed five per cent. of development. Addition of *Arbacia* egg-water to the eggs does not increase the per cent. of those that develop.

2. *Treating the Eggs with Alkali.*—Treatment with alkali before and after insemination gives a high per cent. of cleavage; the eggs, however, in the later stages, particularly the blastula and gastrula, show marked abnormalities as well as a tendency to disintegrate.

On July 7, the following experiment was made three times:

2:40 P.M. Eggs previously procured by straining from ovaries gently cut up in sea-water were divided among seven dishes:

No. 1, uninseminated control in 15 c.c. of sea-water.

No. 2, eggs + 15 c.c. sea-water + 1 drop $n/10$ KOH.

No. 3, eggs + 15 c.c. sea-water + 2 drops $n/10$ KOH.

No. 4, eggs + 15 c.c. sea-water + 3 drops $n/10$ KOH.

2:42 P.M. Nos. 2, 3 and 4 inseminated with freshly made thin *Arbacia* sperm suspension.

2:45 P.M. Nos. 5, 6 and 7. Eggs in 15 c.c. of sea-water inseminated with same *Arbacia* sperm.

2:47 P.M. 1, 2 and 3 drops $n/10$ KOH added to nos. 5, 6, and 7 respectively.

5:00 P.M. Eggs show five per cent. development; in No. 2, much less in 3, 4, 5, 6, 7. Control shows no development.

With shed eggs the results are strikingly different.

July 8, 3:10 P.M. To dry eggs from one female, 100 c.c. sea-water added.

3:20 P.M. To each of seven watch glasses a drop of eggs is added.

No. 1, uninseminated control in 15 c.c. of sea-water.

No. 2, Eggs + 15 c.c. of sea-water + 1 drop $n/10$ KOH.

No. 3, Eggs + 15 c.c. of sea-water + 2 drops $n/10$ KOH.

No. 4, Eggs + 15 c.c. of sea-water + 3 drops $n/10$ KOH.

3:22, Nos. 2–4 inseminated each with one drop of freshly prepared active *Arbacia* sperm suspension.

3:24. Nos. 5, 6, and 7, each with 15 c.c. of sea-water inseminated with one drop of same *Arbacia* sperm.

3:26. To nos. 5, 6 and 7 respectively are added 1, 2 and 3 drops of $n/10$ KOH.

5:00. These eggs show high per cent. cleavage (90 per cent. in no. 4), but also a tendency to lose their membranes when put in normal sea-water. Control, no development.

3. *Insemination with Excess of Sperm.*—The method of heavy insemination succeeds with *Echinarachnius* eggs, giving ten to twenty-five per cent. of cleavage. The later stages are far superior to those eggs in which crossing has been induced through alkali treatment.

July 3, 9:10 A.M. Eggs removed from ovaries divided into three lots—*A*, *B* and *C*. *Lot A* inseminated at 9:15 A.M. with heavy *Arbacia* sperm; *Lot B* similarly at 11:00 A.M.; and *Lot C* similarly at 2:55 P.M. *Lot A* shows 13 per cent. of development at 2:50. *Lot B* 8 per cent. *Lot C* at 5:00 P.M. shows no development.

July 11, 9:30 A.M. 1/2 c.c. of dry *Echinarachnius* eggs placed in 10 c.c. of sea-water. 10:05 A.M., five drops of eggs in each of four watch glasses (Series I.) containing 1, 2, 3 and 4 drops of freshly prepared *Arbacia* suspension. To each of four watch glasses (Series II.) containing five drops of eggs, 1, 2, 3 and 4 drops of *Arbacia* sperm added. Watch glass no. 9 uninseminated control; no. 10, inseminated with *Echinarachnius* sperm.

1:30 P.M. Uninseminated control, no development. Eggs inseminated with *Echinarachnius* sperm 98 per cent. developing. Eggs of both series inseminated with *Arbacia* sperm developing—25 per cent. in most dishes.

These experiments with heavy insemination show that the eggs develop best when inseminated soon after procured; also, that dry eggs are better than "ovary eggs." Whether eggs are added to sperm or vice versa is of no monent, but sperm must be fresh. The eggs treated with dense sperm suspension develop more nearly like the straight fertilized eggs than either the alkali treated or the stale eggs. The larvæ are vigorous and active, swimming to the top as do normal larvæ.[1]

The cross *Echinarachnius* ♀ × *Arbacia* ♂ succeeds far better than the *Arbacia* ♀ × *Echinarachnius* ♂.[2] Indeed in some cases the *Arbacia* egg is in no wise affected by *Echinarachnius* sperm. Thus, we have a sharply defined difference in behavior between the *Echinarachnius* egg and the *Arbacia* egg with respect to foreign sperm.

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[1] Larvæ from this cross were kept for four weeks.

[2] Cf. Gemmil's results with the cross, *Cribrella* × *Asterias*.

B. *Hetero-agglutination in Echinarachnius and Arbacia.*

If fertilizin is necessary for fertilization and if it is likewise specific, we should expect that where cross fertilization is possible in one direction only the sperm agglutination reaction would, if it occurred at all, take place in the same direction The opposite appears to be the case for the *Echinarachnius* × *Arbacia* cross since *Echinarachnius* egg-water has no effect on *Arbacia* sperm while *Arbacia* egg-water has a powerful agglutinating effect on *Echinarachnius* sperm. However, as shown beyond this is not the effect of *Arbacia* sperm agglutinin but is due to a separate substance, a hetero-agglutinin.

1. *Effect of Echinarachnius Egg-water on Arbacia Sperm.*

Sea-water from above *Echinarachnius* eggs highly charged with the substance that agglutinates *Echinarachnius* sperm has no agglutinative action on *Arbacia* sperm. This is true of the most powerful *Echinarachnius* egg-water that was used.· The *Echinarachnius* egg-water does, however, intensely activate *Arbacia* sperm.

Experiments were made with egg-water of both "ovary" and dry eggs.

July 2, 10:00 A.M. Eggs from four *Echinarachnius* strained and washed 5 times in 250 c.c. of sea-water. 2 c.c. of eggs and small bits of ovary. 12:00 M., 210 c.c. of sea-water removed and filtered. Tests with *Echinarachnius* sperm, at 1:30 P.M. and 8:30 P.M., showed great agglutinating strength.

11:00 A.M. Eggs from *Arbacia* washed 5 times in 100 c.c. of sea-water at each washing. 12:05 P.M., 45 c.c. of supernatant sea-water removed and filtered. Tested with *Arbacia* sperm, showed very powerful reaction.

3:00 P.M. *Arbacia* egg-water agglutinates *Arbacia* sperm; *Echinarachnius* egg-water agglutinates *Echinarachnius* sperm, but fails to agglutinate *Arbacia* sperm—trials being made on sperm of three males.

July 10, 10:30 A.M. 1 1/2 c.c. of dry eggs of *Echinarachnius* in 8 1/2 c.c. of sea-water. 11:00 A.M. 8 c.c. of sea-water were pipetted off and filtered. (8 c.c. of fresh sea-water added to the eggs.) 11:05 A.M. 7 c.c. of *Arbacia* eggs from four females put in 50 c.c. of sea-water.

12:10 P.M. *Echinarachnius* secretion gives a 5–6-second reaction with its sperm on 1/500 dilution; full strength, this fails to agglutinate *Arbacia* sperm. *Arbacia* sperm suspensions used were 50 per cent., 20 per cent., 10 per cent. and 1 per cent. freshly prepared in each case. *Arbacia* egg-water agglutinates *Arbacia* sperm.

2:15 P.M. 8 c.c. of sea-water pipetted from the *Echinarachnius* eggs and filtered (8 c.c. of sea-water added to the eggs). 2:00 to 4:00 P.M. This egg-water removed (second washing) gave on *Echinarachnius* sperm a 16-second reaction at 1/12,800 dilution. It failed to agglutinate *Arbacia* sperm but activated them very intensely. *Arbacia* egg-water on these same sperm gave an 8-second reaction on 1/6,400 dilution.

July 16. A strong *Echinarachnius* egg-water, 1/12,800 power, tested on *Arbacia* sperm gave no agglutination, but very intense activation.

The experiments here cited represent but a small percentage of the total number made with *Echinarachnius* egg-water on *Arbacia* sperm. There can thus be no question as to the failure of the *Echinarachnius* egg-water to agglutinate *Arbacia* sperm. On the other hand, the stimulation to increased motility induced by *Echinarachnius* egg-water is very remarkable.[1] In no other case that I know is the activation so marked. It will be noted that the egg-water used is that charged either by thoroughly washed "ovary eggs" or by dry eggs; this, a fact which may be significant, must mean that such sea-water contains very little if any perivisceral fluid.

2. *Effect of Arbacia Secretion on Echinarachnius Sperm.*

Sea-water charged by *Arbacia* eggs with an agglutinating substance for *Arbacia* sperm likewise contains a substance that agglutinates *Echinarachnius* sperm. Certain characters of this agglutination of *Echinarachnius* sperm show that it is due to a hetero-agglutinin distinct from the *Arbacia* sperm agglutinin.

(*a*) *Agglutinative Power of Arbacia Egg-water on Echinarachnius Sperm after Successive Dilution.*—In the first place it may be noted that on dilution of *Arbacia* egg-water the agglutinating substance for *Echinarachnius* sperm drops out before the agglutinin for *Arbacia* sperm. A freshly prepared suspension made of unwashed "ovary eggs" may agglutinate *Echinarachnius* sperm in a very powerful manner, irreversibly so in some cases; but if the egg-water stand or be diluted this effect will gradually disappear, the iso-agglutinin remaining. On the other hand, a suspension of shed eggs not uncommonly gives no agglutinin for *Echinarachnius* sperm, though this class of eggs most actively secrete iso-agglutinin.

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[1] *Asterias* sperm exhibit an interesting phenomenon. When a fresh suspension is prepared, the sperm may be absolutely immobile and remain thus. If now they be mixed with eggs inseminated within one or two minutes previously, they are stimulated to great activity so that they may roll the eggs around.

July 10, 11:05 A.M. 7 c.c. of *Arbacia* eggs from four females in 50 c.c. of sea-water. 12:15, 40 c.c. pipetted off and filtered (40 c.c. of fresh sea-water added to the eggs). 4:00 P.M. *Arbacia* egg-water (first washing) gave an 8-second reaction with *Arbacia* sperm at 1/12,800 dilution. This same suspension gave a 3–4-second reaction on *Echinarachnius* sperm at 1/800 dilution.

July 16, 11:00 A.M. 7 c.c. of *Arbacia* eggs and ovarian tissue put in 50 c.c. of sea-water; strained giving 55 c.c. 1:40 P.M. 37 c.c. of supernatant sea-water pipetted off and filtered, 80 c.c. of sea-water being added to eggs. 2:30 P.M. Supernatant sea-water withdrawn gave at 1/6,400 dilution a 6-second reaction with *Arbacia* sperm; faintly positive for *Echinarachnius* at 1/100 dilution.

(*b*) *Hetero-agglutinin Production of Washed Arbacia Eggs.*— In the second place, if *Arbacia* eggs be repeatedly washed the hetero-agglutinating substance drops out before the iso-agglutinin. A single washing may suffice to remove the hetero-agglutinin. Certainly washing during twelve hours is sufficient entirely to remove the hetero-agglutinin.

July 12, 6:30 P.M. *Arbacia* eggs in 250 c.c. of sea-water; eggs settle at the 7-c.c. mark. 140 c.c. poured off and 140 c.c. sea-water added.

7:30 P.M.—220 c.c. sea-water poured off, 220 c.c. added.
8:30 P.M.—190 c.c. sea-water poured off, 190 c.c. added.
10:00 P.M.—140 c.c. sea-water poured off, 140 c.c. added.
July 13.
8:15 A.M.—210 c.c. sea-water poured off, 210 c.c. added.
9:15 A.M.—150 c.c. sea-water poured off, 150 c.c. added.
10:10 A.M.—200 c.c. sea-water poured off, 200 c.c. added.
11:45 A.M.—240 c.c. sea-water poured off, 240 c.c. added.

At 11:45 A.M. 2 c.c. of eggs left. At 12:30 P.M. this supernatant sea-water gave a 6-second reaction at 1/100 dilution with *Arbacia* sperm. With *Echinarachnius* sperm the last positive reaction made with undiluted supernatant sea-water was at 9:50 P.M., July 12. 8:05 A.M., July 13, test was negative with *Echinarachnius* sperm.

The eggs used July 16 in the experiment cited above were farther washed as follows: 2:35, 3:06 and 5:15 P.M. by removing 90, 94 and 93 c.c. of sea-water, replacing in each case an amount equal to that withdrawn. The last positive effect with undiluted egg-water on *Echinarachnius* sperm was at 3:06 P.M. At 5:15 P.M. the last washing gave a positive reaction with *Arbacia* at 1/1,600 dilution.

In these washing experiments because eggs are lost, it might be argued that the hetero-agglutinin disappears because of the insufficient number of eggs left to charge the sea-water. But since the activity of the hetero-agglutinin in any case is inconstant, its loss in no wise paralleling that of the iso-agglutinin, this argument is scarcely tenable. Nothing is surer than the variation in hetero-agglutinin production by equal bulks of eggs from zero upward.

(*c*) *Precipitation of the Hetero-agglutinin by Echinarachnius Sperm.*—Thirdly, it can be shown that the agglutinin for *Echinarachnius* sperm present in sea-water above *Arbacia* eggs can be precipitated by *Echinarachnius* sperm leaving the *Arbacia* sperm agglutinin practically undiminished in strength. Thus, on July 15, I prepared three vials each with 1 c.c. of filtered *Arbacia* egg-water; to these vials, *A*, *B*, and *C* respectively were added 1, 2 and 4 drops of *Echinarachnius* sperm suspension. A fourth vial, *D* (control), contained 5 c.c. of *Arbacia* egg-water with no sperm. After five minutes the following results on *Echinarachnius* sperm were recorded: *A*, powerful agglutinant undiluted; negative at 1/8 dilution; *B*, positive; *C*, positive.

After ten minutes, *C* was negative; after fifteen *B* faintly positive; at twenty minutes all vials were negative.

Tested on *Arbacia* sperm, the vials *A*, *B* and *D* (control) gave a 6-second reaction at 1/400 dilution.

This experiment when repeated gave essentially the same results: the *Echinarachnius* sperm agglutinin may be completely removed, although this removal is not immediate, the rapidity with which the hetero-agglutinin is destroyed depending upon the density of the *Echinarachnius* sperm suspension used; the iso-agglutinin, on the other hand, remains undiminished in power.

(*d*) *Agglutinative Action of Arbacia Blood on Echinarachnius Sperm.*—Farther, it can be demonstrated that the perivisceral fluid of *Arbacia* has an agglutinative action on *Echinarachnius* sperm though lacking this action on *Arbacia* sperm. Thus on July 17, I carefully collected blood from four *Arbacia* which after clotting was filtered. Ten determinations on *Arbacia* sperm were negative in each case, the sperm showing, however, undiminished response to *Arbacia* egg-water tested after each trial with the blood. This perivisceral fluid agglutinated sperm from six *Echinarachnius* tested in turn.

Frequently, as in another observation on this same day, July 17, females were found whose blood was negative to both *Arbacia* and *Echinarachnius* sperm. Blood is positive with *Arbacia* sperm if the ovaries are ruptured when the animals are opened. Extreme care is necessary to avoid this.

(*e*) *Microscopic Appearance of Echinarachnius Sperm Agglutinated by Arbacia Egg-water.*—Finally, it may be noted that the microscopic appearance of *Echinarachnius* sperm agglutination by *Arbacia* egg-water is markedly different from agglutination by *Echinarachnius* egg-water. On this point I am borne out by four investigators who were good enough from time to time to observe the phenomena. In every instance they declared that they could note a difference in the character of the agglutination. When *Echinarachnius* egg-water is blown into a drop of *Echinarachnius* sperm suspension, the agglutination is sharply defined because after the initial activation the spermatozoa clump, leaving spaces between the clumps practictically free of sperm. After a time, depending upon the strength of the egg-water the clumps break up, the individual sperm being again homogeneously distributed throughout the field. With *Arbacia* egg-water the sperm of *Echinarachnius* behave differently. The agglutinated masses tend to string out with some free sperm among them. The agglutination is slower in beginning and with strong egg-water slow in breaking up, indeed may never break up, the agglutination being permanent. The whole picture suggests a coagulative effect. Agglutination of *Echinarachnius* sperm by *Arbacia* egg-water probably is much like that of *Nereis* sperm by *Arbacia;* this point I attempted to determine but could not succeed in getting *Nereis* sperm at the proper time.

The evidence here presented shows that the iso-agglutinin and hetero-agglutinin found in *Arbacia* egg-water are separate. What the source is, one can scarcely say, although it may well be the blood. Suspensions of eggs thoroughly washed free of perivisceral fluid would, therefore, fail to charge sea-water with the hetero-agglutinin, while the eggs would continue actively to produce the iso-agglutinin. Whatever, the source of the substance, the experiments cited above indicate that it is distinct from the iso-agglutinin and is of the nature of a toxic substance.

C. *Summary of Part III.*

Summarizing we find (1) that cross fertilization between *Echinarachnius* and *Arbacia* is possible but that the *Arbacia* egg is greatly resistant to *Echinarachnius* sperm while the *Echinarachnius* egg is easily fertilized by *Arbacia* sperm. (2) *Echinarachnius* egg-water activates but does not agglutinate *Arbacia* sperm. *Arbacia* egg-water agglutinates *Echinarachnius* sperm. This is a hetero-agglutination by a substance in *Arbacia* egg-water separate from *Arbacia* fertilizin because it may be removed from the egg-water by dilution, by repeatedly washing the eggs and by precipitating it with *Echinarachnius* sperm. It is found in *Arbacia* blood. The microscopic appearance of this toxic hetero-agglutination of *Echinarachnius* sperm is different from that of the iso-agglutination.

IV. Discussion.

Every egg has a fertilizable period during which falls its optimum capacity for fertilization. Thus, *Asterias* eggs normally shed after complete maturation are capable of fertilization on immediate insemination but if procured by placing the ovaries in sea-water these eggs, in the germinal vesicle stage, fertilize only after the maturation process. Apparently, this is true of *Chætopterus* and *Cerebratulus* eggs which come into sea-water in the germinal vesicle stage: insemination is most effective after the germinal vesicle fades and the first maturation spindle is at the mesophase.[1] Immature eggs will not fertilize though sperm may bore into such eggs. Thus, in preparations of the normal fertilization of *Arbacia*, I find many ovocytes with one or more sperm in the cytoplasm, in some cases around the germinal vesicles. Such sperm undergo no change. Moreover, sperm may normally enter immature eggs according to Hempelmann, Shearer and von Hofsten. Sperm entry, therefore, is no criterion of fertilization. Moreover, the fertilizable period of an egg gradually passes over as can be shown by the process of staling, allowing the eggs to stand in normal sea-water. This may take place as in *Platynereis* in an almost incredibly short time; in *Echinarachnius* the time before capacity for fertilization is lost is much longer though not so long as in *Arbacia*. Sperm may

[1] Wilson has found that in *Cerebratulus* after the germinal vesicle has broken down, merogony is possible; enucleated fragments after insemination are not capable of refertilization. (Cf. also Delage.)

enter these stale eggs likewise; for example, in *Platynereis* (Just, '15*b*) sperm not only enter but come into apposition with the female nucleus without inducing development though maturation has been complete. Through the work of Lillie on *Arbacia* these phenomena may be interpreted: immature eggs, stale eggs and fertilized eggs produce no fertilizin; they are incapable of fertilization. One cannot help but postulate a causal connection. Much the same interpretation is possible in *Nereis* and *Platynereis.* In *Echinarachnius* the process is in every way similar to that in *Arbacia.* Whatever prejudice one may have to the fertilizin theory of fertilization because of the immunological terminology, the proved facts one must admit. At the very least, fertilizin is an index to fertilization capacity.

Again, eggs show a period during which they are most susceptible to agents that initiate development. This has been most clearly shown for the starfish by Mathews and by R. S. Lillie. It is generally known that the eggs of some females respond more readily to agents that initiate development than others. In the *Nereis* (Just, '15*a*) one may observe a very striking behavior of the egg to warmed sea-water. From a female quickly dried, dry eggs are taken and divided into two lots. One lot is placed in a small quantity of sea-water at 33° C. and the other into ordinary sea-water. Those eggs in warm sea-water will form jelly, maturate and cleave, the resulting embryos closely simulating the normal. If the eggs in ordinary sea-water be transferred to warmed sea-water *they do not even form jelly.* Nothing is more striking than the comparison between two lots of *Nereis* eggs that have been subjected to warming with and without previous exposure to ordinary sea-water: the dry eggs are in a thick mat of jelly, the washed eggs closely crowded together without any jelly formed. In *Nereis,* then, the egg must have its full content of fertilizin in order to respond to warming as a means of artificial parthenogenesis: it loses its power for initiation of development by artificial means before it loses its fertilizing power. Muscle, nerve and gland respond to artificial stimulation, but the normal mode is most effective. So the sperm is the most efficient activator for the egg. In *Nereis* the best time for artificial parthenogenesis is the time of greatest fertilizin content.

Farthermore, the studies of Lillie have shown that like the fertilized egg, the egg induced to development artificially no longer produces fertilizin. Moore's ('16, '17) studies confirm and extend those of Lillie.

The presence of fertilizin in *Echinarachnius*, *Arbacia* and *Nereis* is indicated by its power to agglutinate specific sperm. But this very agglutination of the sperm is held as an argument against the fertilizin theory because it is held to "inhibit the fertilizing effect of the spermatozoa instead of enhancing it since the cluster formation prevents the sperm from reaching the egg. Even from a teleological viewpoint it is difficult to understand why a substance which only prevents the fertilizing action of the sperm should be a necessary link in such action" (Loeb, '14). First, however, it should be recalled that this agglutination is a reversible process where specific sperm are employed, the clumping lasting from ten to twenty seconds for average egg-water dilution. Second, while Lillie found that agglutination reduces the per cent. of fertilization, Fuchs observed the contrary to be the case. Finally, we should note these agglutinations are made in the laboratory with fairly thick sperm suspension and that not only the motility of the sperm but the density of the suspension are functions in the agglutination reaction. Agglutination would scarcely take place in the sea where the conditions are not those in a drop of water on a slide. As a matter of fact agglutination rarely takes place during insemination in a finger-bowl because we very carefully use thin sperm suspensions to avoid polyspermy. The sperm are thus too widely separated to clump; instead, they stick to the eggs. I, therefore, heartly agree with Loeb when he states that he is "inclined to believe that the cluster formation or agglutination of sperm does not occur when fertilization takes place under natural conditions." But this in no wise invalidates the claims of the fertilizin theory.

Concerning the sperm agglutination by egg-water of another form, hetero-agglutination, it seems to me that it would be necessary to know the *nature* of the agglutination; this demands the microscopic method of studying the agglutination. Thus, *Arbacia* egg-water agglutinates *Nereis* sperm, but this is a toxic agglutination produced by a substance distinct from the iso-agglutinin; it is found in *Arbacia* blood. Likewise the agglutinin in *Arbacia* egg-water for *Echinarachnius* sperm is separate from the iso-agglutinin. These points could be better determined by the microscopic method, by examining the agglutination after diluting the egg-water, attenuating the agglutinin by washing the eggs, etc. The macroscopic method of studying the agglutination would fail. It may be, farthermore, that *Arbacia* blood is generally toxic. Thus, I have found that it will induce jelly-formation, maturation, and if allowed to act too long cytolysis in the *Nereis* egg. We may readily understand that when cross fertilization is possible between forms in one direction only the fact that an agglutination takes place in the opposite direction does not invalidate the fertilizin theory of fertilization if the agglutination is a toxic hetero-agglutination.

Finally, the activation of sperm by egg-water may be of great importance in fertilization as Loeb ('15) points out. Thus, *Echinarachnius* egg-water activates *Arbacia* sperm which are capable of fertilizing *Echinarachnius* eggs. In *Asterias* if at the moment of insemination by immobile sperm more sperm be added they are greatly activated. In *Nereis* agglutination is only possible with very active sperm; such sperm spontaneously form aggregations when mixed with sea-water alone. Sperm that do not form these spontaneous aggregations will not agglutinate. Also in both *Arbacia* and *Echinarachnius* the first stage in the agglutination reaction is activation. Probably in those *Echinarachnius* eggs normally resistant to fertilization there exists a membrane condition that masks the presence of fertilizin. In addition, therefore, to the farther study of activation we must approach the investigation of just such conditions. By these analyses the fertilizin theory may prove of wider application.

LITERATURE CITED.

Delage, Yves

'01 Études experimentales sur la maturation cytoplasmique et sur la parthénogénèse artificielle chez les Echinodermes. Archiv. de Zoöl. Exper. et Gen., Ser. 3, T. G.

Fuchs, H. M.

'14 The Action of Egg-secretions on the Fertilizing Power of Sperm. Arch. Entw'mech., Bd. 40.

Gemmil, Jas. F.

'14 The Development and Certain Points in the Adult Structure of the Starfish, *Asterias rubens.* Phil. Trans., Series B, Vol. 205.

Just, E. E.

'15a Initiation of Development in *Nereis.* Biol. Bull., Vol. 28.

'15b An Experimental Analysis of Fertilization in *Platynereis megalops.* Biol. Bull., Vol. 28.

Lillie, F. R.

'13 Studies of Fertilization, V. The Behavior of Spermatozoa of *Nereis* and *Arbacia* with Special Reference to Egg-extractives. Jour. Ex. Zoöl., Vol. 14.

'14 VI. The Mechanism of Fertilization in *Arbacia.* Jour. Ex. Zoöl., Vol. 16.

'16 History of the Fertilization Problem. Science, N. S., Vol. 43.

Lillie, R. S.

'01 Momentary Elevation of Temperature as a Means of Producing Artificial Parthenogenesis in Star-fish Eggs and the Conditions of this Action. Biol. Bull., Vol. 22.

Loeb, J.

'14 Cluster Formation in Spermatozoa Caused by Specific Substances from Eggs. Jour. Ex. Zoöl., Vol. 16.

'15 On the Nature of the Conditions which Determine or Prevent the Entrance of the Spermatozoön into the Egg. American Naturalist, Vol. 49.

Mathews, A. P.

'01 Artificial Parthenogenesis Produced by Mechanical Agitation. Am. Jour. Phys., Vol. 6.

Moore, Carl R.

'16 On the Superposition of Fertilization on Parthenogenesis. Biol. Bull., Vol. 31.

'17 On the Capacity for Fertilization after Initiation of Development. Biol. Bull., Vol. 32.

Wilson, E. B.

'03 Experiments on Cleavage and Localization in the Nemertine Egg. Archiv. Entw'mech., Bd. 16.

THE FERTILIZATION REACTION IN ECHINARACHNIUS PARMA.

III. The Nature of the Activation of the Egg by Butyric Acid.

E. E. JUST.

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I. Introduction.

The egg of *Echinarachnius* as shown elsewhere (Just, '19*a*) following engulfment of one sperm immediately becomes immune to farther sperm entry. This change takes place in the cortex *before* the membrane lifts off and probably represents the neutralization of the fertilizin by the anti-fertilizin present in the egg. This is the mechanism for the prevention of polyspermy, and not the immediately succeeding membrane lifting. Loeb, however, found that butyric acid, though it be capable of calling out "fertilization membranes" in one hundred per cent. of the eggs of the sea-urchin, is none the less incomplete in its action, for if by shaking he removes these "fertilization membranes" sperm penetrate the eggs, membranes form anew, and development proceeds like the normal. This finding certainly does not support his view that fertilization is a kind of artificial parthenogenesis for after fertilization eggs or fragments thereof do not "refertilize" (see older work of Driesch and the important work of Wilson on *Cerebratulus*). The present paper reports results of an attempt to determine whether or not in *Echinarachnius* eggs activation by butyric acid is complete in the sense that it is complete when membranes form after the initial stage in the fertilization process. If we assume that activation is complete in eggs that have formed membranes after butyric acid then hypertonic sea-water exposure should give the best development with these eggs. Any other assumption is absurd unless the use of butyric acid is unnecessary, when used with hypertonic sea-water, for the best type of development. Again, on the assumption that with membrane formation following butyric acid the activation is complete, as it is in fertilization, the egg should react on insemination as do fertilized eggs or fragments thereof. The observations show that after butyric acid treatment under optimum conditions eggs form membranes (Part II.); and that in such eggs activation is complete since with hypertonic treatment they give rise to cleavages, gastrulæ and plutei of a high degree of normality, and since they fail to develop after insemination (Part III.).

II. Activation of Echinarachnius Eggs by Butyric Acid.

Eggs of *Echinarachnius* if properly exposed to butyric acid in sea-water are activated and so form membranes that are indistinguishable from those formed following insemination.

A. *Optimum Exposure to Butyric Acid.*

Without the experiments being cited it may be said that by using various mixtures of butyric acid in sea-water the best mixture was found to be 2 c.c. of *n*/10 butyric acid plus 50 c.c. of sea-water. The optimum exposure for this mixture discovered by removing samples of eggs from the butyric mixture at five second intervals, is thirty-five seconds. In some cases eggs were exposed for sixteen minutes.

The following experiment of July 8 is cited as typical: 9:50 A.M., 15 dishes each with 250 c.c. of sea-water. 10:18 A.M., 2 c.c. *n*/10 butyric acid plus 50 c.c. of sea-water thoroughly mixed. 10:20 A.M., shed eggs from one very fine female exposed to butyric mixture, samples being removed at intervals to dishes of 250 c.c. of normal sea-water. Table I. gives the data.

TABLE I.

No.	Length of Exposure.	Per Cent. of "Full Membranes."
1	20 seconds	14
2	25 "	72
3	30 "	85
4	35 "	93
5	40 "	87
6	45 "	83
7	2 minutes	0
8	5 "	0
9	6 "	0
10	7 "	0
11	8 "	0
12	9 "	0
13	10 "	0
14	11 "	0
15	12 "	0

Eggs of nos. 7–15 showed a tendency to go bad; this increased with the length of exposure, for no. 15 eggs were very bad.

The results are essentially the same in the experiments of July 11 and July 26 as shown in Table II.

TABLE II.

No.	Length of Exposure.	Per Cent. of "Full Membranes."	
		July 11.	July 26.
1	20 seconds.	3	18
2	25 "	39	36
3	30 "	50	76
4	40 "	96	91
5	45 "	67	83
6	50 "	49	—[1]
7	55 "	12	—[1]
8	60 "	1	1
9	65 "	0	—[1]

[1] No exposure for this length of time.

During August 4, seven lots of eggs from different females were exposed to 2 c.c. $n/10$ butyric acid plus 50 c.c. sea-water for 20, 25, 30, 35, 40, 60, 90 and 120 seconds. The 35-second exposure in each case gave 91, 90, 98, 93, 99, 97 and 97 per cent. full membranes. Observations of August 6 were about the same.

These citations are sufficient, I think, to show that there is a very definite optimum exposure between rather narrow limits for the activation of *Echinarachnius* eggs by the mixture of butyric acid and sea-water employed. But if the eggs are exposed to *higher* concentrations of butryic acid even for a shorter time they do not form full rounded membranes that stand off from the cortex but thick membranes adhering closely to the cytoplasm. Thus, on July 6, eggs exposed to 3 c.c. of $n/10$ butyric acid plus 50 c.c. of sea-water and those exposed to 3.5 c.c. of the acid plus 50 c.c. of sea-water for 20 seconds each gave one hundred per cent. of these thick contracted membranes. These membranes are entirely different from the so-called "fertilization membranes" got with lower concentrations of butyric used. Apparently, Loeb obtained the same results with *Arbacia* eggs. Speaking of the effect of butyric on this egg he says: "When transferred to sea-water, they did not form a conspicuous fertilization membrane as did the eggs of *S. purpuratus* under the same circumstances, but only a fine gelatinous layer which was not easily visible" (page 71).

On shaking some time after removal from butryic sea-water full membranes partially collapse; contracted membranes on shaking break and the eggs give off buds. These buds show extreme variations in size. Butyric treatment alone (at least for the longest exposure tried, sixteen minutes) never gives cleavage. The eggs never go beyond the monaster stage.

B. *Condition of the Eggs as a Factor in the Response to Activation by Butyric Acid.*

Without exception my best results with *Echinarachnius* eggs have been obtained with dry shed eggs—*i. e.*, eggs deposited by the animals in clean dry watch glasses. It is not always practicable to wait until the animals shed; I, therefore, proceed as follows: The animals are washed in sea-water then in running tap-water, shaken dry and placed aboral side down after a slight cut around the peristome which usually suffices to induce shedding;[1] such eggs are scarcely inferior to normally shed eggs. In the experiments cited above the butyric mixture previously prepared was added directly to the Syracuse glass containing the eggs. I was content to work with relatively a small number of eggs always from but one female.[1] The amount of butyric mixture used, therefore, could be likewise small (in my experiments never more than 12.5 c.c.) so that the eggs could be thoroughly stirred up and still settle quickly with the result that when at most the 1 to 1.5 c.c. was removed the amount of acid carried over was negligible.[2]

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[1] Great care was exercised to keep everything sterile. On only one occasion was an egg found in the two cell stage in the control—not more certainly than one in three or four hundred eggs, but the experiment was discarded.

1. *Effect of Washing.*—Dry eggs may be washed quickly once or twice in a small amount of sea-water without affecting the butyric treatment. Additional washings will cut down the per cent. of butyric membranes and washings extending over one to two hours will result in the failure of butyric acid to form membranes. Eggs similarly treated may be still capable of fertilization. Eggs incapable of fertilization never respond to butyric acid treatment.

An experiment of July 26 shows the difference between dry and washed eggs:

8:55 A.M. A fine lot of eggs divided among three dishes—*A*, *B* and *C*. Lot *A* dry; Lot *B* washed three times in fifty minutes; to lot *C* two drops of body fluid added.

10.00 A.M. Lot *B* gave the following results after exposure to butyric sea-water:

Exposure in seconds	20	25	30	35	40	60
Per cent. of membranes	6	19	38	27	14	—1
Lot *A* (unwashed) per cent. of membranes	18	36	76	91	83	1

On August 4 similar results were obtained. After four washings in thirty minutes the per cent. of membranes formed was reduced one half after optimum exposure as compared with the unwashed.

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[1] Indeed, in my judgment the practise of using large quantities of eggs from a number of females is a most pernicious one, introducing as it does more unknown factors. Nothing is so variable as the echinid egg, as Tennent, Medes and Goldfarb have shown—the work of the last-named being particularly concerned with our problem; in a lot of eggs from several females one must get a very heterogeneous population—especially, as is usually the case, if the worker obtains his eggs by mincing the ovaries in sea-water.

[2] Transfers were sometimes made to 4,000 c.c. of normal sea-water; the results obtained were no better than those to 250 c.c. of sea-water.

Against these observations the objection might be raised that the failure of washed eggs to form membranes in greater number is simply due to the low concentration of butyric acid in sea-water since from the washed eggs all the water cannot be removed. Against this we have (1) the fact that dry eggs quickly washed once or twice may give one hundred per cent. membranes. If, by chance, body fluid was present with shed eggs they were always washed. (2) After washing all but a negligible amount of water can be removed. (3) Diminution in concentration of acid does not explain absolute loss by the egg of the power to respond to butyric acid which may take place in an hour. (4) Finally, washed eggs subjected to butyric acid in higher concentration (*e. g.*, 3 c.c. *n*/10 butyric plus 50 c.c. of sea-water) if they form membranes at all form the thick closely adherent membranes which result from exposure to too great concentration. Washing alone is responsible for the failure of the eggs to form membranes; repeated washings in 10 to 15 c.c. of sea-water for one to two hours or a single washing in 250 c.c.[1] will render eggs incapable of activation by butyric.[2]

2. *Effect of Body Fluid.*—My 1914 experiments on the initiation of development of *Nereis* eggs through increased temperature proved that serum as definitely inhibits initiation of development, as it does fertilization (Just, '15). I was, therefore, led to study the effects of *Echinarachnius* body fluid on butyric acid activation of *Echinarachnius* eggs. Eggs mixed with body fluid form few if any full membranes; they usually form the thick closely adherent type of membrane.

(*a*) Thus, the eggs of Lot *C* of July 26 mentioned on page 43 (eggs plus two drops of body juice) gave the following:

Exposure in seconds	20	25	30	35	40	60
Per cent. of membranes	0	3	14	16	11	0

(*b*) July 15, 10:30 A.M. Eggs plus body fluid (2 drops each) in butyric acid sea-water for 20, 25, 30, 35 and 40 seconds.

10:50 A.M. *Not one full membrane;* some eggs have the thick contracted membranes.

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[1] I did not wash in the larger quantities often, for with them the difficulty of recovering the small number of eggs increases; moreover, concentrating the eggs takes time—thus introducing another factor.

[2] Washing may not have the same effect on *Arbacia* eggs which are much more resistant. (See Just, '19*b*.)

(*c*) July 8. Eggs shaken from ovaries into butyric mixture gave the following results:

Exposure in seconds	20	25	30	35	40	45	50	55
Per cent. of membranes	33	38	34	48	31	20	17	0

All contracted membranes.

(*d*) Experiments of August 4 and 6. Serum eggs form no membranes after butyric acid exposure.

In (*d*) the cortical changes that are responsible for membrane formation were probably complete (see beyond). The actual lifting off of the membrane is not a *sine qua non* of activation. (See Just, '19*b*. Loeb, '15, discusses this point apropos certain observations of Brachet.)

Heilbrunn found that in *Arbacia* eggs membrane elevation by acid is inhibited by *Arbacia* blood.

C. *Summary of Part II.*

Summarizing we find that butyric acid in sea-water activates *Echinarachnius* eggs if they are in best physiological condition; slight washing will not impair their capacity to respond to the acid but long washing will. The body fluid inhibits membrane elevation.

III. Analysis of Butyric Acid Activation.

If membrane elevation is a sign of complete activation those eggs exposed for optimum length of time to sea-water with butyric acid in optimum concentration should respond best to subsequent hypertonic treatment. Moreover, activation by butyric if complete as in fertilization should inhibit fertilization. The present section deals with the analysis of butyric acid activation and contains the evidence that shows that such activation is complete.

A. *Effect of Hypertonic Sea-water on Butyric Treated Eggs.*

Butyric acid acting along on *Echinarachnius* eggs (at least for exposures employed which were up to 16 minutes) never induces cleavage. But after butyric activation hypertonic sea-water causes cleavage—62 per cent. in one case—which is scarcely to be distinguished from the normal; such eggs form gastrulae and develop into plutei that swim to the top of the dishes. Mixtures in the proportions 5, 6, 7 and 8 c.c. of 2.5 M NaCl plus 50 c.c. of sea-water in each case were employed. The best results were obtained with the mixture of salt and sea-water in the proportions of 5 cc.. of 2.5 M. NaCl plus 50 c.c. of sea-water.[1] The procedure was as follows: Eggs exposed to butyric acid sea-water were removed at intervals of 5 seconds to 250 c.c. of sea-water. Following about 20 minutes in sea-water eggs were carried over to dishes of hypertonic sea-water at 15, 20, 25, etc., minutes. Twenty-five minutes treatment is best. *Eggs previously exposed to butyric acid for 35 seconds (optimum exposure for membranes) gave the highest per cent. of cleavage and the best type of development.*

It would be tedious to cite individual experiments on this point. The reader would scarcely be interested in experimental details on the rather threadbare subject of the effect of hypertonic sea-water following butyric activation. It is, of course, generally understood that hypertonic sea-water is most successful subsequent to butyric acid treatment where membranes have been formed. There would certainly be no reason to use butyric acid if this were not true. Notwithstanding all this, I wish to emphasize that those eggs with the highest per cent. membranes (optimum exposure) yield best to hypertonic treatment. Eggs under- or over-exposed to butyric acid do not give the same type of development.[2]

B. *Effect of Insemination Following Butyric Acid Activation.*

After removal to normal sea-water following optimum exposure to butyric acid sea-water *Echinarachnius* eggs form 100 per cent.

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[1] The 2.5 M. NaCl solution was always freshly made. No solution was ever older than six hours when added to the sea-water.

[2] The successfully treated eggs are certainly very beautiful; as far as the gastrula stage in most cases they appear to be perfectly normal. The plutei develop more slowly than those from fertilized eggs and are not so hardy. Possibly, with even nicer treatment, the development could be improved.

Cytological study of eggs induced to develop with artificial means has been made by Wilson, Hindle and Herlant. In the living *Echinarachnius* egg we observe that while in sea-water following butyric acid treatment a monaster forms beyond which stage the egg does not develop. Having been properly exposed to hypertonic sea-water the egg forms in sea-water a complete astral system. Overexposure to hypertonic sea-water results in the formation of innumerable asters when the egg is removed to sea-water: such eggs fail to cleave or form very irregular cleavages.

membranes in highly successful cases. Such eggs mixed with sperm do not develop though the membranes be previously removed as soon as formed. Thus, activation with butyric acid is complete; eggs with membranes have undergone an irreversible change.

The Experiments.—The method followed in these experiments is simple. Eggs are exposed to *n*/10 butyric acid in sea-water (2 c.c. of acid plus 50 c.c. sea-water) and removed to 250 c.c. of normal sea-water at five second intervals; these dishes comprise Series *A*. From the dishes of series *A* eggs are removed, shaken as soon as membranes are formed, and inseminated with fresh sperm suspension; thus, series *B* is established. Samples of eggs from each of the *A* dishes inseminated without shaking off the membranes make up series *C*. The membranes formed in series *A* and later the cleavages in *B* and *C* are recorded; *B* and *C* dishes are then gently washed in fresh sea-water and set aside for study of the development during the next twenty-four hours.

The experiment of July 9 is typical: 10.00 A.M. 6 dishes each with 250 c.c. of sea-water to which eggs are removed after exposure to butyric sea-water after 30, 35, 40, 60, 120 and 180 seconds (series *A1* to *A6*). Samples from *A1* to *A6* shaken and inseminated (series *B*, 1 to 6). Series *C* samples from *A1* to *A6* inseminated without shaking. Table III. gives the results:

TABLE III.

No.	Exposure in Seconds.	Per Cent. of Membranes in *A*.	Per Cent. of Cleavages,		Per Cent. of Swimmers.	
			In *B*.	In *C*.	In *B*.	In *C*.
1	30	70	27	28	7	6
2	35	76	30	27	6	3
3	40	74	29	23	0	1
4	60	5	13	3	2	4
5	120	0	30	43	44	41
6	180	0	48	44	41	39

B4 to B6 and C4 to C6: cleavages very irregular; "swimmers" extremely abnormal—exogastrulæ, microgastrulæ, blastulæ with dense opaque interiors evidently due to abortive gastrulation.

I have given this particular experiment because not a very high per cent. of the eggs formed membranes. This ought, therefore to be crucial, for since under-exposed eggs (*i. e.*, those which are do not form membranes after short exposures) may fertilize perfectly we may assume that these particular eggs were on the border line of proper exposure. Insemination, however, caused no membranes (cf. Herbst) in the over-exposed eggs, and this is always the case, though some of the eggs cleave.

Membranes once formed may be removed, but after insemination these eggs develop no farther than uninseminated butyric treated eggs. Study of the figures given in Table III. shows first, that the cleavages of unshaken inseminated eggs (with membranes) and of shaken inseminated eggs (without membranes) are approximately equal; and second, that the per cent. of membranes in *A* plus the per cent. of cleavages in *B* or *C* is very close to a hundred. This must mean that eggs with membranes formed with butyric acid are not fertilizable. In a given lot of eggs exposed for the optimum time to butyric some are not completely activated and have not formed membranes. These are the eggs that cleave following insemination; the removal of the membranes does not affect the per cent. of cleavages thus obtained.

The experiment of July 8 part of which has already been given (p. 40) is clearly decisive. Following exposure to butyric sea-water Series *A* eggs were shaken and inseminated (series *B*) with the results found in Table IV.

TABLE IV.

No.	Exposure.	Per Cent. of Membranes (Series A).	Per Cent. of Cleavage After Insemination (Series B).
1	20 seconds.	14	50
2	25 "	72	26
3	30 "	85	15
4	35 "	93	6
5	40 "	87	16
6	45 "	83	19
7	2 minutes	0	41
8	5 "	0	43
9	6 "	0	—
10	7 "	0	—
11	8 "	0	—
12	9 "	0	—
13	10 "	0	—
14	11 "	0	—
15	12 "	0	—

B7 to B15: cleavages very abnormal; frequently incomplete many spindles present.

July 26, August 4 and August 6. Eggs after 35 seconds exposure showed 100 per cent. membranes but failed to develop following insemination whether freed of membranes or not.

Early in the season eggs were found that failed to form a single membrane subsequent to optimum butyric treatment. Such eggs on insemination never developed. Eggs inhibited by the body fluid may not form membranes; if they be inseminated they do not form membranes or otherwise develop. Such eggs have undergone cortical changes but membrane formation is suppressed. In serum eggs the suppression usually is not complete: the membrane thickens and remains stuck to the egg.

Moore working with *Arbacia* found that those eggs that form butyric membranes do not develop after insemination though the sperm may penetrate. Whether under similar conditions sperm enter the eggs of *Echinarachnius* I am unable to say. Mere penetration, however, in no wise affects our position. Development does not depend on sperm penetration simply; development begins with certain cortical changes which having been initiated by agents other than the sperm inhibit action by superimposed sperm.

C. *Summary of Part III.*

We may summarize the results of this section as follows: Butyric acid eggs with membranes respond best to hypertonic sea-water giving development that closely simulates the normal. Eggs with butyric membranes cannot be fertilized though the membranes be removed. These facts indicate that cortical activation is complete.

IV. Discussion.

1. Workers generally appreciate how capricious is the response of eggs to agents that initiate development. Often the procedure of a given experiment may be repeated with altogether different results. Hence we conclude that the varying "physiological condition" of the eggs is responsible for our results. Study of fertilization in *Echinarachnius* (Just, '19*b*) shows that this physiological condition depends on the presence of fertilizin: eggs that have a high fertilizin index show a high per cent. of

development; eggs washed free of fertilizin lose their fertilizing power; immature eggs and fertilized eggs show no fertilizin reaction and are incapable of fertilization (and re-fertilization).

Now, development of sea-urchin ova and of marine ova generally by chemical and physical agents is of course purely artificial; it is at least debatable that these agents are as efficient for initiating development as specific sperm. In *Nereis* and in *Echinarachnius* the volume of evidence is incontrovertible: only those eggs in best condition (as shown by their fertilizin tests) respond to artificial agents that initiate development. If eggs from a given lot fail of fertilization the remainder of that lot will not respond to artificial agents. Indeed, in both *Nereis* and *Echinarachnius* the egg through washing—and loss of fertilizin—loses its power to respond to warming (*Nereis*) or to butyric acid (*Echinarachnius*) before it loses its power to respond to sperm. Eggs with full fertilizin content most nearly ensure success with artificial agents.

Blood inhibits fertilization—a truism to the embryologist; where marine ova are normally shed and insemination takes place in the sea, it is generally found best to wash the eggs before insemination. Normally shed eggs always give a high per cent. of development; eggs cut or washed from the ovaries are not so good. Thus, in *Podarke* and in *Cumingia*, for example, eggs develop in greatest number if laid. The blood blocks fertilization "by occupying the ovophile group of the fertilizin, thus preventing action of the latter upon the egg by union with egg receptors" (Lillie). It probably acts in the same way in artificial initiation of development.

In *Nereis* and in *Echinarachnius* blood inhibits both fertilization and artificial initiation of development. In *Arbacia* Lillie found that blood inhibits fertilization while Heilbrunn observed that it also inhibits membrane formation. The worker, therefore, who studies "artificial parthenogenesis" is between Scylla and Charybdis—either he will not wash the eggs and thus have blood inhibitor present, or he will wash too much and so bring about too great secretion of fertilizin. This may seem fanciful to some but experience with *Nereis*, *Platynereis* and *Echinarachnius* teaches that the egg is anything but an inert cell; for successful treatment with artificial agents it must be handled with extreme care to insure uniform results.

2. *Echinarachnius* eggs that form membranes through butyric acid treatment are completely activated since such eggs respond to subsequent hypertonic sea-water treatment with development that closely simulates the normal, and since eggs under- or over-exposed to butyric acid and which do not form membranes never form membranes following hypertonic sea-water treatment nor do they develop at all normally. Activation, again, must be regarded as complete since butyric acid eggs with membranes fail to develop after insemination even though the membranes be removed as soon as formed; in this they resemble fertilized eggs or fragments thereof that fail to re-fertilize. Finally, these butyric acid treated eggs with membranes fail to give the fertilizin test as rapidly as do fertilized eggs. We must, therefore, conclude that activation is as complete in eggs with butyric membranes as in eggs with fertilization membranes.

It is interesting to note that Loeb found that his sea-urchin's eggs after butyric acid membrane formation were capable of fertilization provided he had removed the membranes by shaking. Lillie, however, found that *Arbacia* eggs after membrane formation by butyric acid cease to produce fertilizin as do fertilized eggs. Moore confirmed Lillie's butyric observation on *Arbacia* and also discovered that eggs with butyric membranes are incapable of fertilization. To Loeb, complete membrane formation by butyric is an incomplete activation; to the workers with *Arbacia* and *Echinarachnius* eggs membrane formation by butyric acid is a complete activation. In initiation of development, therefore, the egg gives a similar response both to the artificial agent and to the spermatozoa. This, however, does not entail subscription to the famous "lysin theory."

It is now some twenty years since the work on artificial parthenogenesis began. During this time eggs have been subjected to every kind of agent imaginable, a vast amount of literature has been produced, and many guesses have been made on the basis of this work as to the nature of the fertilization reaction. This work on artificial parthenogenesis constitutes one of the important chapters in the history of modern biology and no mean part among the many brilliant achievements of Loeb which aside from their inherent value have another claim to distinction—their stimulating effect on the new experimental zoölogy. But *artificial parthenogenesis is not fertilization.* And it is indeed unfortunate that the term artificial parthenogenesis ever had that connotation. Thus we read of "fertilization membranes" by this or that chemical, "fertilizing agents," "chemical fertilization," etc., when we know that there are eggs that will respond to very slight changes in the environment. The eggs of *Amphitrite*, *Asterias*, *Nereis*, etc., for example, will respond simply to agitation. Artificial parthenogenesis *per se* is too significant to masquerade as fertilization. The processes initiated in artificial parthenogenesis and fertilization probably are the same. But to argue that the sperm carries a lysin because a host of agents activate the egg is bad logic. It is far simpler to postulate that the egg contains the necessary mechanism for development. Such a postulate embraces not only artificial parthenogenesis and fertilization, but also the widely occurring and *real* parthenogenesis. Artificial parthenogenesis teaches us that the egg is a highly irritable cell that will respond to various agents; it has placed us but little nearer a solution of the fertilization problem than we were twenty years ago. One might as easily deduce from the contraction obtained on a curarized muscle with any one of a number of stimuli the nature of the nerve impulse as to formulate a theory that since CO_2, or any other of a number of agents induces development fertilization is due to a lysin carried by the sperm.

Nor yet have purely morphological hypotheses proved satisfactory. The Boveri hypothesis as its predecessors is no longer considered adequate; and such deductions as those of Meves lead only to absurdities.

Lillie's assumption that the egg in its fertilizable condition contains a substance (fertilizin) which needs merely to be activated is logical and is based on observation and experiments which themselves, theory aside, excite admiration. Future work will determine how much this theory has helped toward a solution of the fertilization problem.

LITERATURE CITED.

Heilbrunn, L. V.

'15 Studies of Artificial Parthenogenesis. II. Physical Changes in the Egg of *Arbacia.* Biol. Bull., Vol. 29.

Just, E. E.

'15 Initiation of Development in *Nereis.* Biol. Bull., Vol. 28.

'19a. The Fertilization Reaction in *Echinarachnius parma.* I., Cortical Responses of the Egg to Insemination. Biol. Bull., Vol. 36.

'19b. II., Rôle of Fertilizin in Straight and in Cross Fertilization. Biol. Bull., Vol. 36.

Lillie, F. R.

'14 Studies of Fertilization. VI., Mechanism of Fertilization in *Arbacia.* Jour. Ex. Zoöl., Vol. 16

Loeb, J.

'13 Artificial Parthenogenesis and Fertilization. The University of Chicago Press.

'15 Concerning Brachet's Ideas of the Rôle of Membrane Formation in Fertilization. Biol. Bull., Vol. 28.

Moore, C. R.

'16 On the Superposition of Fertilization on Parthenogenesis, Biol. Bull., Vol. 31.

THE EFFECTS OF A DIET OF POLISHED AND OF UNPOLISHED RICE UPON THE METABOLIC ACTIVITY OF PARAMECIUM.[1]

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The purpose of the following experiments was to discover the effects of the presence and absence of the vitamin contained in rice upon the metabolic activity of *Paramecium*. It was successfully proved by Funk (2), that the vitamin which prevents the deficiency disease known as beri-beri in man is removed from the rice by the process of polishing. Since the lack of this vitamin in the diet of human beings produces a seriously diseased condition it seemed a promising subject for research to determine whether or not the effects could be so far-reaching as to alter the metabolism of a one-celled organism, *Paramecium*. Calkins (1) had previously shown that pancreatic vitamin, a specific in the case of marasmus, had no influence whatever upon the metabolic activities of *Paramecium*. Calkins used the division rate as an index to the vitality of the organism, believing that certain substances in the culture medium may increase the rate of division and others decrease it. This criterion is the one used in the following investigation.

The experiments were performed upon individuals from five pure lines, all from wild cultures started in the laboratory at the same time and kept in spring water and hay infusion for one month. The pure lines were started on December 4, 1917. Each individual was isolated in three drops of distilled water and one drop of 1 per cent. malted milk solution, renewed every other day. The malted milk was prepared by pouring 50 c.c. of boiling distilled water over .5 gram of Horlick's malted milk. After several divisions had taken place each pure line was transferred from the depression slide to a large stock dish containing 200 c.c. of distilled water and 5 c.c. of malted milk solution. Once a week one half of the stock solution was removed and an equal amount of fresh water and 5 c.c. of fresh malted milk were added.

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[1] From the Biological Laboratory of Bryn Mawr College.

In order to eliminate as far as possible the influence of unknown variables every effort was made to prevent contamination of the culture media; graded pipettes were used in measuring the media and the slides containing the individuals were kept in a moist chamber at constant temperature, and removed for observation at approximately the same hour every day. Individuals for the special problem were isolated on March 9, 1918, three being taken from each pure line. Of these three, one was isolated on a depression slide in two drops of distilled water and two drops of 1 per cent. rice water made from polished rice, and a third in two drops of distilled water and two drops of 1 per cent. rice water made from unpolished rice. The rice water was prepared by boiling .5 gram of finely ground rice in distilled water and making up the solution to 1 per cent. after evaporation. The number of divisions was noted at the end of every twenty-four hours, and one individual from each group was isolated on a clean slide in the same medium as before. While the bacterial content is a questionable factor in the experiments, it is controlled to a large extent by the sterilization of slides and pipettes, by the boiling of all media and by the daily isolation of the individuals in fresh media.

Some trial experiments were made for perfection of technique, and then, using eleven days as a fair unit of time, final averages were made of the rates of division in each group. The individuals in malted milk showed the normal rate of division which was slightly higher than that in hay infusion. Peebles (8) has shown that if rightly used a weak solution of malted milk is a most satisfactory culture medium. The metabolic activities of the individuals in polished and in unpolished rice exhibited the influence, not only of the presence and absence of the vitamin, but also of the change of environmental conditions as represented by a new culture medium. Results are given in the accompanying tables.

The number of divisions during the first twenty-four hours of experimentation are not given in the tables, because some time must be allowed for adaptation to new conditions.

Table I.

The Daily Rate of Division.

Line.	Medium.	2d Day.	3d Day.	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.
1	Polished rice	0	0	1	0	1	0	1	0	0	0
1	Unpolished rice	1	0	0	1	1	0	1	0	0	1
1	Malted milk	1	1	1	1	1	1	1	1	0	1
2	Polished rice	0	1	0	0	1	0	1	0	1	0
2	Unpolished rice	0	1	1	1	1	1	1	1	0	1
2	Malted milk	1	1	1	1	1	1	1	1	1	1
3	Polished rice	0	1	0	1	0	0	1	0	1	0
3	Unpolished rice	1	0	0	0	2	0	1	1	0	1
3	Malted milk	0	1	0	1	0	2	1	1	1	1
4	Polished rice	0	1	0	1	0	0	1	0	0	0
4	Unpolished rice	0	1	1	0	1	0	0	1	0	0
4	Malted milk	0	1	0	1	1	1	1	1	0	1
5	Polished rice	1	0	0	1	0	0	1	0	0	0
5	Unpolished rice	1	0	1	1	0	0	1	0	1	1
5	Malted milk	1	1	0	1	1	0	0	1	1	2

Table II.

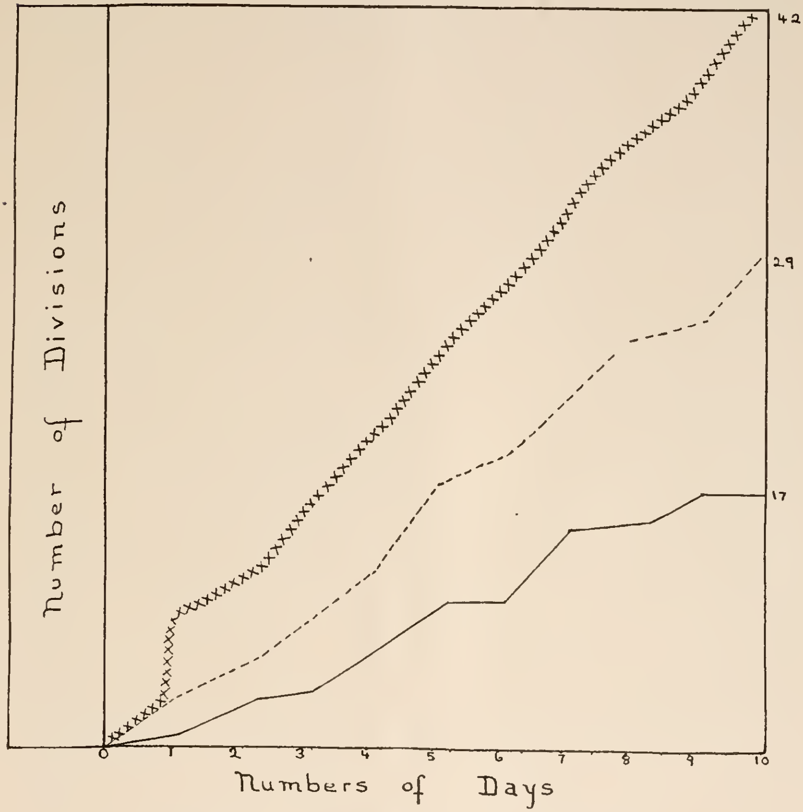
Total of Divisions in Each Group and Averages.

Medium.	Total of Divisions.	10-day Average.	Daily Average.
Polished rice	17	3.4	0.34
Unpolished rice	29	5.8	0.58
Malted milk	42	8.4	0.84

The graph shows that the division rate with polished rice is broken and irregular, while that of the unpolished rice is fairly regular and that of the malted milk very regular in daily increase in the number of divisions. From these results it is evident that unpolished rice is much less favorable than malted milk as a culture medium for *Paramecium*, and that polished rice is quite inadequate for maintaining the vitality of the organisms. It seems probable that the essential factor lacking in the diet of polished rice is the vitamin removed in the process of polishing.

According to McCollum and Davis (4) polished rice is deficient in fat-soluble A, and water-soluble B, the accessory food substances necessary for normal growth and maintenance of life. The fats of rice contain very little if any fat-soluble A, while all the water-soluble B is carried in rice polishings. In using polished rice as a diet for young rats McCollum and Davis found it necessary to supplement the diet with water-soluble and fat-soluble vitamines. They found that 2 per cent. skim milk powder in combination with the rice supplied nearly enough water-soluble B for normal growth. In the experiments with *Paramecium* the addition of one drop of 1 per cent. malted milk solution to one drop of 1 per cent. polished rice water and two drops of distilled water restored the division rate to the normal

TABLE III.



GRAPH SHOWING DAILY TOTAL FOR EACH GROUP.

Crosses indicate divisions of individuals in malted milk. The dotted line indicates divisions of individuals in unpolished rice. The continuous line indicates divisions of individuals in polished rice.

rate shown in malted milk alone. The efficacy of malted milk is due to the presence in it of both water-soluble B and fat soluble A. Unpolished rice contains both but not enough fat-soluble A for maintenance (6).

Since orange juice is so potent a specific in the treatment of deficiency diseases in man, a second set of experiments was undertaken to see if when used with polished rice the accessory food substances in orange juice would be an adequate substitute for the rice vitamin. Parallel experiments were tried with unpolished rice and malted milk to determine whether or not orange juice would increase the metabolic activities of the organism in media already known to be adequate for growth and maintenance.

The orange juice was prepared by boiling 1 c.c. of orange juice in distilled water and making up the solution to 1 per cent. after evaporation. Previous trial experiments had been made with different percentages of the orange juice, 5 per cent., 1 per cent., 2 per cent., and 3 per cent., and malted milk. The results indicated that the 1 per cent. solution would be most favorable for work with *Paramecium.* The races used for the special problem were isolated from various sources, Pure line A was identical with Pure line 1 of the first experiments, Pure line B and Pure line C were isolated from cultures obtained from Powers and Powers, Lincoln, Neb., and the University of Pennsylvania respectively, Pure line D from a wild culture just introduced into the laboratory, and Pure line E from a mixed culture kept in the laboratory in hay infusion all winter. These gave a variety of physiological conditions upon which to experiment. The individuals in Pure lines B and C were much larger and more vigorous than those from the other lines. The wild race was especially small but very vigorous after the firm establishment of the line, while the races which had been kept in the laboratory all winter were small and sluggish. Following the precautions observed in the previous experiments, four individuals from each line were isolated on April 22, 1918, one in two drops of distilled water and one drop of 1 per cent. malted milk solution, a second in one drop of distilled water and two drops of 1 per cent. malted milk solution, a third in one drop of distilled water, one drop of 1 per cent. malted milk solution and one drop of 1 per cent. orange juice, and a fourth in two drops of distilled water and one drop of 1 per cent. orange juice.

The work was carried on for eleven days, at the end of which time averages were made from the rates of division in each group. The two control groups showed the influence of different degrees of concentration upon the division rate, and the group in orange juice and water was used to determine whether or not orange juice served alone as an adequate food substance. Results are shown in the accompanying tables.

Table IV.

The Daily Rate of Division.

(*a*) 2 drops of distilled water and 1 of malted milk.
(*b*) 1 drop of distilled water and 2 of malted milk.
(*c*) 1 drop of distilled water and 1 of malted milk, and 1 of orange juice.
(*d*) 2 drops of distilled water and 1 of orange juice.

	2d Day.	3d Day.	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.
Line A.										
(*a*).....	2	0	1.5	1	2	1	2	2	1	1.5
(*b*)....	2	1	0	1	1	2	2	1	2	0
(*c*)......	2	2	1	2	1	2	2	2	1	1
(*d*).....	1	0	1	1	dead					
Line B.										
(*a*).....	2	1	2	1	1	2	1	0	1	2
(*b*)......	2	1	1	1	2	1	1	1	1	1
(*c*)......	2	1	1	1	1	2	1	0	1	1
(*d*).....	0	0	dead							
Line C.										
(*a*).....	2	1	1	2	1	1	0	1	1	1
(*b*)......	2	1	1	2	1	2	1	1	1	1
(*c*)......	2	1	2	2	1	1	2	1	1	2
(*d*).....	1	0	0	1	0	dead				
Line D.										
(*a*)......	1	1	2	2	2	1	1	1	1	2
(*b*)......	1	0	2	1	2	2	2	2	1	2
(*c*)......	1	2	0	1	3	2	2	2	1	0
(*d*).....	1	1	1	0	dead					
Line E.										
(*a*).....	1	1	1.5	1.5	0	0	2	2	0	2
(*b*)......	2	1	1.5	1.5	0	1	2	1	1	2
(*c*)......	1	1	2	2	0	1	2	1	0	2
(*d*).....	1	1	1	dead						

The small variations in division rate are within the limits of experimental error, with the exception of group (*d*). From these facts it is to be concluded that orange juice is not an adequate food substance, that it does not increase the metabolic

TABLE V.

TOTAL OF DIVISIONS IN EACH GROUP AND AVERAGES.

Line.	*A.*	*B.*	*C.*	*D.*	*E.*	Total.	10-day Average.	Daily Average.
(a)......	14	13	11	14	11	63	12.6	1.26
(b).......	12	12	13	15	13	65	13	1.3
(c).......	16	11	15	14	12	68	13.6	1.36
(d)......	3	0	2	3	3	11	2.2	0.22

activities of organisms in malted milk, that the slight variation in the concentration of media employed in these experiments does not alter the rate of division and finally that these conclusions are applicable to races representing a divergence of previous physiological conditions.

TABLE VI.

THE DAILY RATE OF DIVISION.

(a) 2 drops of distilled water and 2 of polished rice water.

(b) 2 drops of distilled water and 2 of unpolished rice water.

(c) 2 drops of distilled water and 2 of polished rice water and 1 of 1 per cent. orange juice.

(d) 2 drops of distilled water and 2 of unpolished rice water and 1 of 1 per cent. orange juice.

	2d Day	3d Day.	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.
Line A.										
(a).....	0	0	0	1	0	0	0	dead		
(b)......	0	1	1	0	0	1	0	0	0	0
(c)......	0	0	0	0	0	0	0	dead		
(d).....	1	0	0	0	0	0	0	0	0	0
Line B.										
(a).....	0	0	0	0	1	0	0	dead		
(b)......	0	2	0	0	1	0	0	0	0	dead
(c)......	1	0	0	1	0	0	0	0	0	dead
(d).....	1	0	0	1	0	0	0	0	0	0
Line C.										
(a).....	0	0	0	0	0	dead				
(b)......	0	0	1	0	0	0	dead			
(c)......	0	0	0	0	0	0	0	0	dead	
(d).....	0	1	0	0	0	0	1	0	dead	
Line D.										
(a).....	0	0	0	0	0	dead				
(b)......	0	0	1	0	0	dead				
(c)......	0	0	0	0	0	0	0	0	0	0
(d).....	0	0	0	0	1	0	0	0	0	0
Line E.										
(a).....	1	0	0	0	dead					
(b)......	0	1	0	0	1	dead				
(c)......	1	0	0	1	dead					
(d).....	1	0	0	0	1	0	1	0	0	1

TABLE VII.

TOTAL OF DIVISIONS IN EACH GROUP AND AVERAGES.

Line.	*A.*	*B.*	*C.*	*D.*	*E.*	Total.	10-Day Average.	Daily Average.
(*a*)......	1	1	0	0	1	3	0.6	0.06
(*b*).......	3	3	1	1	2	10	2.0	0.2
(*c*).......	0	2	1	1	2	6	1.2	0.12
(*d*)......	1	2	2	1	4	10	2.0	0.2

The experiments with rice and orange juice were begun May 6, by isolating four individuals from Lines A, B, C and D, two as controls in two drops of distilled water and two drops of 1 per cent. rice water, from polished and unpolished rice respectively, and two like the controls with the addition of one drop of 1 per cent. orange juice to each. The work was carried on for eleven days at the end of which time averages were made of the rates of division in each group, to determine whether or not the substitution of the accessory food substances in orange juice for the rice vitamin had increased the metabolic activities of the individuals in polished rice, and also to note any stimulus to division produced by the addition of orange juice to unpolished rice. The results are shown in the accompanying tables.

It is evident from these results that all of the *Paramecia* were in a state of depression which was not altered to any appreciable extent by the presence of orange juice, although the individuals in unpolished rice and orange juice seem to have had more resistance to the unknown factors which produced dissolution in the other groups. The orange juice had a slightly stimulating effect upon the metabolic activities of the organisms in polished rice but absolutely no effect whatever upon the division rate of those in unpolished rice. From these results it may be concluded that orange juice has not a powerful influence upon the metabolic activities of *Paramecium*.

Much appreciation is due Dr. Florence Peebles for her helpful supervision of this work.

LITERATURE.

1. **Calkins, G. N., and Eddy, Walter H.**
   '16–'17 The Action of Pancreatic Vitamin upon the Metabolic Activity of Paramecium. The Proceedings of the Society of Experimental Biology and Medicine, Vol. 14.
2. **Funk, C.**
   '12 Journal of State Medicine, Vol. 20, p. 341.
   '13 Über die physiologische Bedeutung gewisser bisher unbekannter Nahrungbestandteile, der Vitamine. Ergeb. d. Physiol., Vol. 13, pp. 125–205.
3. **Hargitt, G. T., and Fray, W. W.**
   '17 The Growth of Paramecium in Pure Cultures of Bacteria. Journal of Experimental Zoölogy, Vol. 22.
4. **McCollum, E. V., and Davis, M.**
   '15 The Nature of the Dietary Deficiencies of Rice. Journal of Biological Chemistry, Vol. 23, p. 181.
5. **McCollum, E. V., and Kennedy, C.**
   '16 The Dietary Factors Operating in the Production of Polyneuritis. Journal of Biological Chemistry, Vol. 24, p. 491.
6. **McCollum, E. V., and Pitz, W.**
   '17 The Vitamine Hypothesis and Deficiency Diseases—A Study of Experimental Scurvy. Journal of Biological Chemistry, Vol. 31, p. 229.
7. **McCollum, E. V., and N. Simmonds.**
   '17 A Biological Analysis of Pellagra-Producing Diets. The Value of Some Seed Proteins for Maintenance. Journal of Biological Chemistry, Vol. 32, p. 347.
8. **Peebles, F.**
   '12 Regeneration and Regulation in Paramecium caudatum. Biol. Bull., Vo . 23, p. 2.

GLYCOGEN IN THE CHICK EMBRYO.

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In his paper, "Glycogen in the Nervous System of Vertebrates"[1] Gage has pointed out that glycogen is not found in such great abundance in the organs of the chick at any one period of its development as in the embryonic organs of many other forms, including mammals; from which the inference is at once drawn that this difference is correlated with the nature of development in the hen's egg, where the yolk furnishes an abundant supply of food, available at any moment for the needs of the embryo. The development of the various organs is a gradual one as compared with that of forms which are provided with less yolk, and glycogen, the builder of tissue and energy producer, appears only as it is needed in the successive stages.

This is particularly striking when comparing the chick with such forms as *Petromyzon* and *Amblystoma*, studied by Gage. When contrasted with mammals the same explanation is not as valid, for the mammalian tissue is also able to develop gradually, calling upon the maternal supply when necessary. However it may be supposed that in mammals the embryo is less directly in contact with its food supply than in the chicken. A great amount of glycogen could not be produced as quickly when a demand arose in tissue development, and consequently a greater supply is produced and maintained in the embryonic tissue itself.

The following observations in regard to the occurrence and distribution of glycogen in the tissue of the chick, from primitive streak stages to the age of about ten days, are based on material which was fixed and stained according to the methods given in Gage's article, and in all cases the presence of the glycogen has been verified by testing the sections with saliva, according to Gage's directions.

It gives me pleasure to be able to acknowledge my great indebtedness to Dr. H. L. Wieman, under whose direction this problem was undertaken, and whose assistance, interest and criticism made it possible to carry out the work.

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[1] *Jour. Comp. Neur.*, Vol. 27, No. 4.

Perhaps the most striking fact observed in the formation of glycogen in the chick was its almost invariable presence in the yolk-sac, from the earliest stages to chicks of ten days. Fig. 1 (Plate I.) shows globules of glycogen in the yolk-sac of a chick of six somites, before the vascular area has developed. In these earlier stages the glycogen is located in the yolk-sac near the embryo, and it is found more or less consistently distributed throughout the vascular area when this has been formed, more abundant near the embryo than towards the edges. It gives to the walls of the blood-vessels of this region a striking red color and under high power appears as mahogany-colored masses scattered among the cells. Fig. 2 shows a part of the vascular area of a six-day chick with the glycogenated areas represented in stippling in the walls of the blood-vessels. Figs. *2a* and *2b* show parts of the vascular areas of seventy-two-hour and four-day chicks respectively, under higher magnification. In Fig. *2b* the actual globules of glycogen are represented in black. Apparently the yolk-sac furnishes a way-station in which carbohydrates are stored as glycogen on their way from the yolk to the embryonic tissue.

This formation of glycogen in the yolk-sac makes an interesting comparison with the formation of glycogen in the placenta of mammals, a fact which seems well established. In mammals the maternal glycogen-forming tissues are perhaps relieved in in this way of the strain of a sudden call for large amounts of sugar. In both cases a supply of glycogen is on hand ready-formed and able to be called upon quickly.

In the earliest chick embryos observed there appeared to be, here and there, a trace of glycogen in the yolk itself in the form of globules somewhere near the site of the embryo, but this fact is not at present established beyond a doubt in my mind, although it would accord with what Gage has observed in the eggs of the *Petromyzon* and *Amblystoma*.

In embryos of from fifteen to twenty-four hours glycogen was found in the ectoderm of the head region, both in the developing nervous system and in the ectoderm below the headfold (Fig. 3).

As soon as the heart tissue begins to develop (at about twenty-eight hours) it takes on its glycogenic function and from that time to the oldest embryos studied the heart is the most consistently and by far the most abundantly glycogenated organ of the body. The glycogen here appears in very deeply stained granules embedded close together throughout the muscular walls of the entire heart, and extending into the sinus venosus and the bulbus. Fig. 4 shows, in stippling, the position of the glycogen in the heart tubes of a chick of nine somites. Fig. 5 shows a part of the heart of a six-day chick, with glycogen abundant in the muscles. Fig. 5*a* represents a part of the wall of a four-day chick's heart, and Fig. 5*b* of a ten-day chick's heart, under high power. In both of these the actual globules of glycogen have been represented in black.

The glycogen of the vascular area seems to be continued under the embryo with more or less regularity. Certainly as the gut is formed glycogen extends into it, appearing in some cases very distinctly at the opening of the gut onto the yolk, more abundant at the opening of the hind-gut than of the foregut, extending in a thirty-five-hour chick into the pharynx, and from fifty-six hours on being very noticeable in the intestine. Fig. 6 shows the fore-gut of a four-and-a-half-day chick just in front of the anterior portal. The glycogen in the entoderm of the gut and below it is stippled. Fig. 7 is the posterior intestinal portal of a seven-day chick, and Fig. 7*a* is the intestine and splanchnic entoderm of the same a few sections posterior to Fig. 7. Fig. 8 is a transverse section through the intestine of a ten-day chick, showing glycogen abundant in the epithelium.

At fifty-six hours, and in all later stages, glycogen was found in the myotomes, being particularly striking in those sections where the body was cut sagitally, the glycogen here appearing like mahogany-colored beads on the spindle-shaped myotomes (Fig. 10, Plate II.). Fig. 9 (Plate II.) is a transverse section of a chick of five days showing the position of the glycogen in the myotomes. Under high power one of these myotomes appeared as in Fig. 11, where the actual spots of glycogen are represented. As other muscular parts develop the myotomes become less glycogenated, but they still retain this function to some extent.

In a seventy-two-hour chick there was an appearance of glycogen in the mesoderm between the two portals and the two sides of the future body-cavity, but as this did not seem consistent with other stages, its presence, or the normality of its presence, is rather doubtful.

In a ninety-six-hour chick a faint trace of glycogen was found in the septum medullæ. At four and a half days the glycogen was very distinct here and it remained in this position up to the oldest stage studied, making a very striking mahogany-colored streak along the floor of the medulla (Figs. 12 and 13). From five days on it was found in a similar position along the floor of the spinal cord in its lumbar and sacral regions (Fig. 14).

At this same age (five days), it appeared in the ectoderm of the anal plate.

At approximately six days glycogen appeared in some of the muscular tissue around the eyes, forming strings of beads along the fibers of the tissue. This became more and more abundant in later stages (Figs. 15 and 16).

At an age of from seven to eight days it was observed in other parts of the head region—in the muscular tissue below the tongue, and medially in a clearly defined area of developing cartilage which formed a keel-like structure below the brain and between it and the pharynx. (Fig. 17).

At this age also glycogen occurs in the muscular tissue along the sides of the body, and at a slightly older stage it appears in a small patch of ectoderm in the nose region.

At ten days it is found in the cartilage of the head and body quite generally. Fig. 18 shows a trace of it in a developing centrum in the head region. In Fig. 14 it appears in the vertebral cartilage of the body. Fig. 19 shows it very abundant in the cartilage of one of the developing limbs. Fig. 19*a* shows the same under high power. The glycogen of the cartilage appears as very distinct red spherules which practically fill the cells.

Glycogen is found in great abundance at this age in the muscular tissue, not only in that already mentioned, but also in the more superficial muscles of the head, in the pectoral muscles (Fig. 20), in the muscles of the limbs (Fig. 19), and in fact in practically all of the musculature of the body. In some places it seemed to occur in the deeper layer of the skin.

It appeared at this age also in a mass of nerve cells lying in the dorsal fissure of the spinal cord in its lumbar region (Fig. 14). Its occurrence in these cells was also noted by Gage.

These results are perhaps more significant in a negative than a positive way. They show that in the chick the storage of glycogen in the embryonic tissue itself is not as extensive as in other forms, and that it does not become a well-developed function of the embryo in its earliest stages, though glycogen does appear in some of the tissue and becomes more and more abundant in later stages. The embryo depends for its energy production and building material upon the food of the yolk, a part of which is kept on hand in the form of glycogen in the yolk-sac.

The majority of the drawings were made with a 16 mm. objective and 4x eye-piece. For those marked high power a 4 mm. objective, 4x eye-piece were used. All drawings were made with a camera lucida.

EXPLANATION OF PLATE I.

FIG. 1. Yolk-sac of a six-somite chick before development of the vascular area. Glycogenated cells in black.

FIG. 2. Splanchnopleure of the vascular area of a six-day chick with glycogen in the walls of the blood-vessels (stippled). *bl. v.*, blood-vessels; *cœl.*, cœlome; *y.s.*, yolk-sac.

FIG. 2*a*. Vascular area of a seventy-two-hour chick under high power. (Glycogen stippled.)

IG. 2*b*. Vascular area of a four-day chick, high power. Actual masses of ogen represented in black.

FIG. 3. Glycogen (stippled) in the ectoderm of a chick with six somites. *ect.*, ectoderm.

FIG. 4. Glycogen (stippled) in the heart tubes of a chick with nine somites. *ht.*, heart; *ph.*, pharynx.

FIG. 5. Heart of a six-day chick with glycogen abundant in the muscular walls. (Stippled.) *l.a.*, left auricle; *l.v.*, left ventricle; *r.a.*, right auricle; *r.v.*, right ventricle.

FIG. 5*a*. Wall of a four-day chick's heart under high power. The black portions represent glycogen.

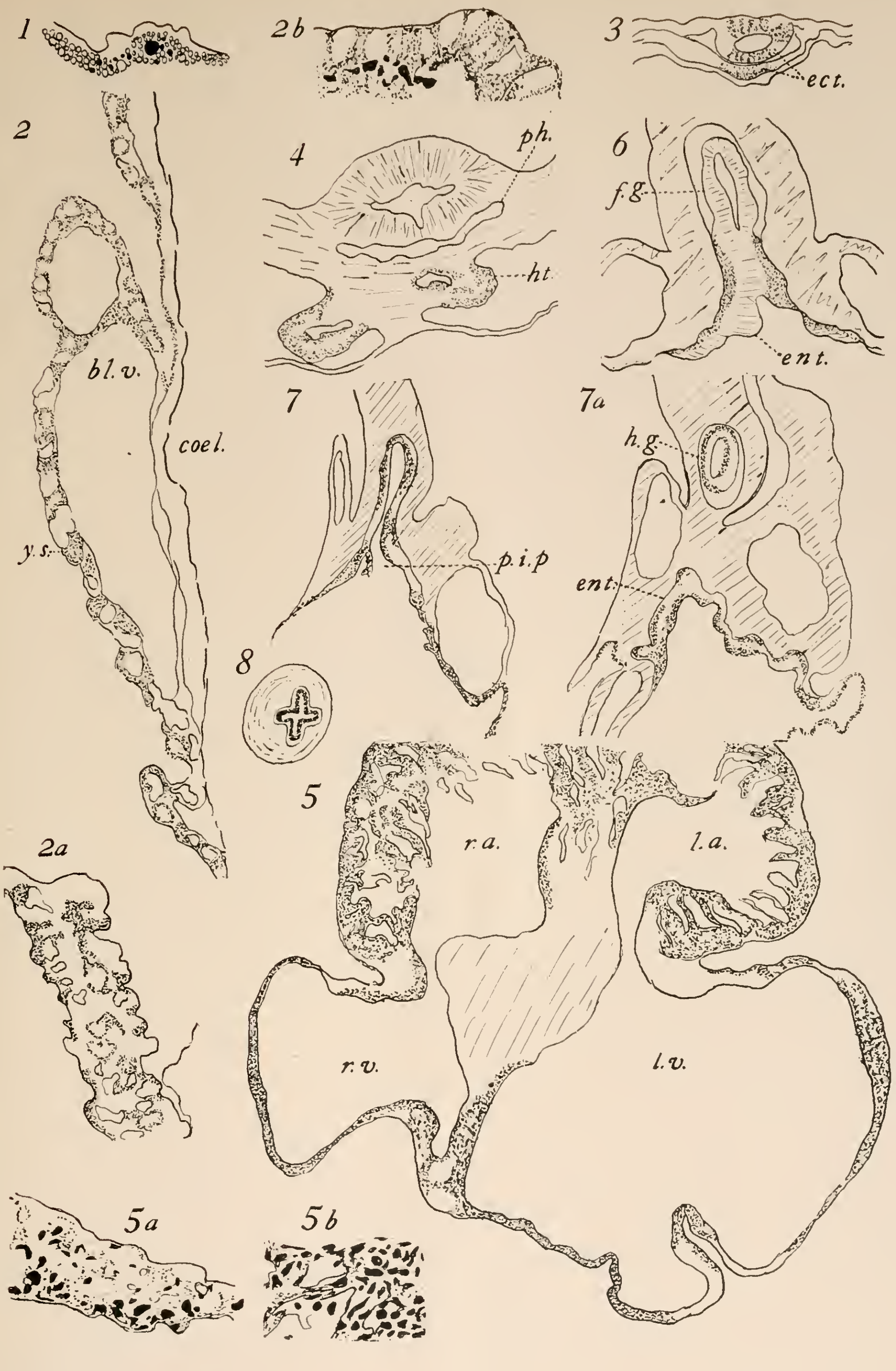
FIG. 5*b*. Ten-day chick's heart. High power. Globules of glycogen in black.

FIG. 6. Fore-gut of a four-and-a-half-day chick just anterior to the anterior portal. (Glycogen stippled.) *ent.*, entoderm; *f. g.*, fore-gut.

FIG. 7. Posterior intestinal portal of a seven-day chick. (Glycogen stippled.) *p.i.p.*, posterior intestinal portal.

FIG. 7*a*. Hind-gut and splanchnic entoderm of the same, a few sections posterior to 7. (Glycogen stippled.) *ent.*, entoderm; *h.g.*, hind-gut.

FIG. 8. Cross section through intestine of a ten-day chick. (Glycogen stippled.)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l.a.
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l.v.
5a
5b

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Explanation of Plate II.

Fig. 9. Transverse section of a five-day chick showing glycogen (stippled) in the myotomes and intestine. *m.y.*, myotomes; *int.*, intestine

Fig. 10. Sagittal section through the posterior end of a seven-day chick showing glycogen (stippled and dotted) in the myotomes. *m.y.*, myotomes.

Fig. 11. A myotome in sagittal section. Five days. High power. The black areas are glycogen.

Fig. 12. Transverse section of the medulla oblongata of a six-day chick. (Glycogen stippled.) *m.*, medulla; *s.m.*, septum medullæ.

Fig. 13. Sagittal section of the septum medullae. Six to seven days. (Glycogen stippled.) *c.*, cord, *s.m.*, septum medullæ.

Fig. 14. Transverse section of spinal cord (lumbar region) of a ten-day chick, showing glycogen (stippled) in the cartilage of the vertebra, the floor of the cord and a mass of nerve cells in the dorsal fissure. *ca.*, cartilage; *n.*, glycogenated nerve tissue.

Fig. 15. Glycogenated muscle near the eye of a six-day chick. (Glycogen stippled.) *dien.*, diencephalon; *no.*, optic nerve; *o.*, eye.

Fig. 16. Glycogenated muscles near the eyes of a seven-day chick. (Abbreviations as in 15.)

Fig. 17. Glycogenated cartilage of developing skull. Seven to eight days *ca.*, cartilage; *dien.*, diencephalon; *o.*, eye; *ph.*, pharynx.

Fig. 18. Developing centrum in the head of a ten-day chick, showing glycogenated area in stippling. *ca.*, cartilage.

Fig. 19. Glycogenated cartilage and muscles of the foot of a ten-day chick. (Glycogen stippled and dotted.) *ca.*, cartilage; *m.*, muscles.

Fig. 19*a*. Cartilage of the same under high power.

Fig. 20. Pectoral muscles and cartilage of a ten-day chick.

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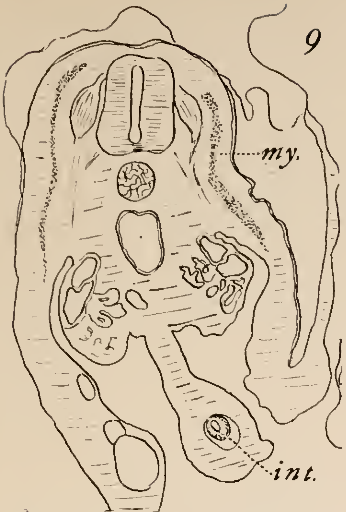

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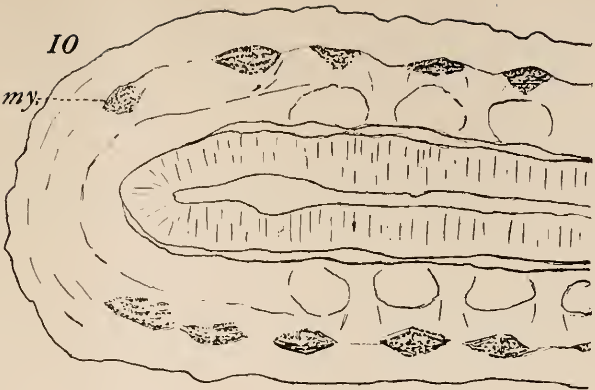

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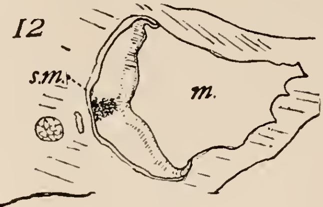

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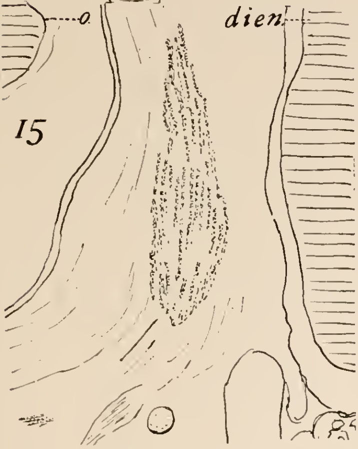

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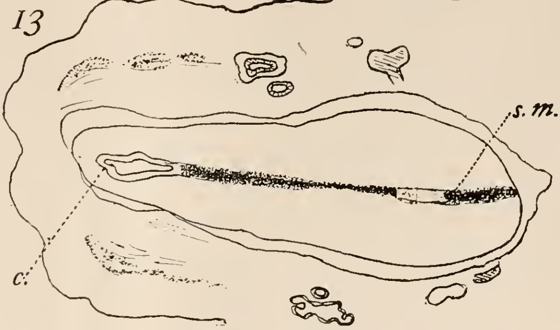

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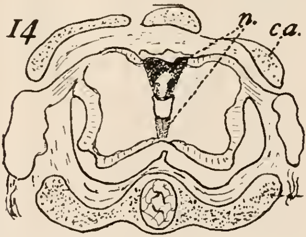

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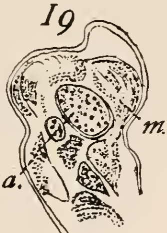

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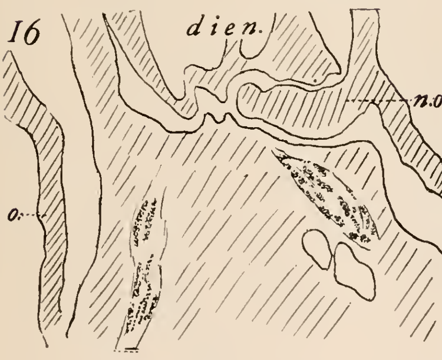

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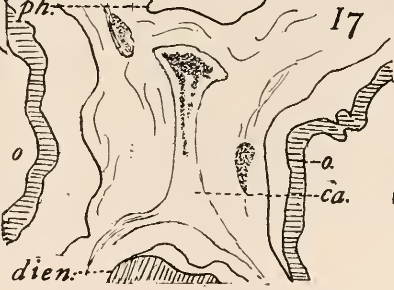
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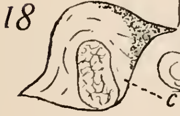

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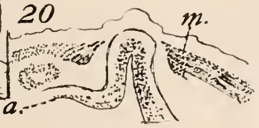

H. JEANETTE ALLEN.

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BIOLOGICAL BULLETIN

ON THE PRESENT STATUS OF THE CHONDRIOSOME-PROBLEM.[1]

J. DUESBERG.

Before I come to the subject of this address, I want to make a short incursion into the domain of nomenclature. As I recently pointed out, the word "mitochondrium" designates a body of granular form. Since those who use it as a general denomination have in mind certain bodies which very often appear as filaments, they are led to speak of "filamentous mitochondria," something which is inconceivable. Besides, using a term in a new sense adds greatly to the confusion already existing in the nomenclature, much more than the use of a new term. In 1915 I reverted to the use of the word "chondriosome," for although there may be some objection to it, in the present state of things it has two distinct advantages—one, that it does not preclude anything as to the form of the bodies which it is supposed to designate; the other, that it is already extensively used, so that its further adoption would bring us very near to that much-to-be-desired unification in our nomenclature.

Speaking of the present status of the chondriosome-problem, I have to speak of what has been done in this particular field of cytological research, and also of what is still to be done. There is much to say on either topic, since the number of observations published on chondriosomes is enormous and since an agreement on some the most fundamental questions is as yet far from being reached. Owing to lack of time, however, my account must necessarily be confined to a few essential points.

Beginning with what has been accomplished, one of the most gratifying results for the cytologist who works in that field is the following. After the publication of Benda's somewhat complicated method, it was a common opinion that what that method brings into evidence is only a distorted appearance of the cellular structures. Thus, however, were disregarded a number of observations, some of which belong to the earliest period of cytological investigation: I shall limit myself to mention von Brunn's observations on the formation of the spiral filament in the spermatozoön of the mouse, von la Valette St. George's work with dahlia-violet applied in vivo, Michaelis's discovery of the peculiar properties of the janus-green.[1] Quite lately the chondriosomes have been extensively studied in the living cell, especially in tissue cultures: prominent investigators in this field are W. H. and M. R. Lewis. These recent observations have confirmed what the old ones already taught—that chondriosomes are not artefacts and that they appear in the fixed and stained preparations very much the same as they are in the living cell.

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[1] Lecture delivered at Woods Hole Marine Biological Laboratory, Mass., July 23, 1918.

Another important result of the recent studies on chondriosomes is a simplification in our conception of the morphology of the protoplasm. When Benda first described his "mitochondria" (at that time, he was convinced that the granular form was the rule), he thought he had discovered a new cellular organule. The question soon arose: what is the relationship between these organules and other constituents of the cell, such as Flemming's fila, Maggi's plastidules, Altmann's bioblasts, Boveri's archoplasmic granules, etc.? Is there any relation between these bodies or are they entirely different? In the last supposition, the structure of the cytoplasm appeared as something so complicated that many, especially under the influence of Fischer's experiments, cleared the field by designating as an artefact any structure which appeared after fixation. We know now, principally as the result of Meves's exertions, that all these elements are one and the same thing. Flemming's filaments (at least those which he described in the resting cell, in contradistinction to the mitome of the dividing cell), Maggi's plastidules found by that author and by the brothers Zoja, his pupils, in practically all tissues, Altmann's bioblasts whose discovery unfortunately led their author to theoretical considerations which threw disrepute on his observations, Boveri's archoplasmic granules which in the dividing egg of *Ascaris megalocephala* surround the centrosphere,—all these things are identical with Benda's mitochondria and with our chondriosomes. We know further that the morphology of that same substance in the different cells, or even in a given cell at different periods of its life, is subject to great variations. Thus the controversy between the partisans of an exclusively filamentous structure of the protoplasm and those who believed only in granules, has come to an end. The morphological variations of chondriosomes in one cycle of life are especially conspicuous in the seminal cells of many invertebrates. There, in many species, the chondriosomes of the spermatogonia are granules; in the spermatocytes, the granules flow together and build up filaments and finally in the spermatid, a further coalescence very often takes place, the result of which is the formation of a single, compact, bulky body, well-known as von la Valette St. George's "Nebenkern." A reverse process is also observed. To return to an example already given, Boveri's archoplasmic granules in the dividing egg of *Ascaris* are derived from bodies which have, in the young ovocyte, a filamentous form. One should be warned however against reuniting all protoplasmic structures in one single group, as some have done. As far as we know, there is no relationship between the chondriosomes and the idiosome, nor between the chondriosomes and most of the elements designated as Golgi's apparatus. It is becoming more and more clear in my opinion that the latter denomination should be reserved to that element which in most cells, especially in young cells and during the period of rest, is closely connected with the idiosome and which has been described under various names, such as Centrophormien, Zentralkapseln, formazioni periidiozomici, idioectosome, etc. If I had to express in a schema the structure of a young resting cell, I would propose this one, (s. next p.) which in my opinion, corresponds to the present state of our knowledge. It should be added here that, for the majority of investigators, including myself, the chondriosomes belong to the cytoplasm. Quite recently, the question of their nuclear origin has been brought up again by Schreiner. Taking for granted that Schreiner's description be accurate, nothing proves however that his interpretation be correct, for the process described as an expulsion of substance from the nucleus might just as well be the reverse. And even if we accept Schreiner's interpretation, we find that the question of the origin of the chondriosomes has really not been touched, since chondriosomes are present in all cells before the process which Schreiner describes takes place.

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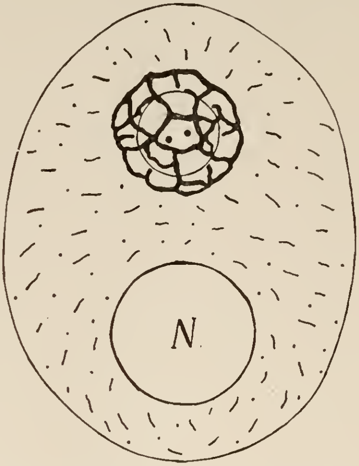
[1] Probably the reason why janus-green has until lately been so little in favor among cytologists is that a number of dyes delivered under that name are either some other product, or a substance mixed with impurities, which may be toxic.



N, nucleus; above, idiosome with centrioles, surrounded by the apparatus of Golgi. In the protoplasm, chondriosomes.

Finally, the tremendous amount of work published in recent years has revealed the presence of chondriosomes in practically all cells, animal and vegetal. The only animal cells that constitute an exception are the superficial cells of the epiderm: that is, cells whose protoplasm is entirely differentiated and transformed into a new substance. Whether chondriosomes exist in the adult red blood-corpuscle might perhaps be questioned also. It is not impossible that the presence of hæmoglobin, which is precipitated by the fixing reagents, be responsible for the negative results obtained by most authors. But, one may well ask, how can the cytologist decide whether he is in presence of chondriosomes or not? A number of criteria may be applied in this connection: the behavior towards preserving reagents and dyes; or better still, the evolution of a given cell-constituent, as for instance in the seminal cells; or the continuity of a given structure from the embryonic cell to the adult stage. If however our objector means to say that it may be difficult in a given case to decide whether or not a certain structure is a chondriosome, then I heartily agree with him, but I fail to see therein a reason for general scepticism. Rather it should prove a stimulus for further investigation. It should not be forgotten that the chondriosomes are by no means the only structures that may be hard to identify in certain instances. Indeed, sometimes we have great difficulty in recognizing a nucleus. Take for example the controversy on the so-called Blochman's nuclei in the egg of certain insects, or (as a personal illustration) the divergence of opinion between Retzius and myself as to what part of the spermatozoön of *Ciona* is the nucleus. In this very field (spermiogenesis) the same difficulty is met with constantly when we attempt to study atypical forms.

I come now to the second part of this address: what is still to be done in the investigation of the chondriosomes? Since brevity is essential, I shall limit myself to the consideration of two questions, both of great importance *i. e.*, the rôle played by the chondriosomes in the process of differentiation and their rôle in fertilization. Meves was the first to express the opinion that the chondriosomes of the embryonic cell represent an indifferent material, susceptible of various differentiations in the different tissues. Since that time a number of papers supporting this idea have been published, of which I shall only mention those on the development of the collagenous fibrils (Meves), of neurofibrils (Hoven) and of myofibrils (Duesberg). The same idea is also and very strongly supported by a number of observations concerning the processes of differentiation in vegetal cells. My own observations on myofibrillogenesis have found confirmation in different quarters and so far no specific criticism has been expressed. On the other hand, the conclusions concerning the chondriosomal origin of the neurofibrils and of the collagenous fibrils have been criticized, respectively by Cowdry and M. R. Lewis. I frankly admit that Cowdry's arguments are worth being very carefully considered, but I should perhaps be allowed at the same time to venture the question whether there is really such a problem as the origin of neurofibrils; in other words, whether the neurofibrils really exist in the living cell. As to the fibrils of the connective tissue, a detailed criticism of M. R. Lewis's paper is out of place here, but I want however to point out that little effort has been made by that author to demonstrate that the fibrils appearing in her tissue-cultures are collagenous fibrils. Yet, I gather from Baitsell's observations that great care should be taken in interpreting such fibrillar structures. Personnally, I have not found in my recent investigations on development, normal and regenerative, any reason for changing my mind. I can clearly see however, how interesting it would be to apply the experimental method to the solution of these problems: experiments on regeneration of tissues, on centrifuged eggs, on the behavior of cells under abnormal and pathological conditions and under the influence of poisonous substances, etc., a field which so far has been explored very little.

The second question I want to consider is this: are the chondriosomes an idioplasmic substance? This opinion was first expressed by Benda. It found strong support in Meves's discovery of chondriosomes in all embryonic cells and later in that author's description of the behavior of the male chondriosomes in *Ascaris* during fertilization. The question is so important that it should be considered with greatest care and at some length.

At the basis of the hypothesis of the idioplasmic nature of the chondriosomes is the idea in which the protoplasm plays a rôle in the transmission of hereditary characteristics. This opinion has always been that of a number of biologists, although a minority, and it has been supported by experimental researches, namely by the well-known experiments made by Godlewsky. The question then arises: what part of the spermatozoön represents the protoplasmic idioplasm? It cannot be the axile filament, which is not constant and is obviously an organ of motility; it cannot be the headcap, nor the acrosome, since these are also inconstant, in some cases (marsupials) only transitory parts of the spermatozoön; nor do I see that any argument can be brought forth in favor of the idioplasmic nature of the centrioles, or of the small amount of protoplasmic ground-substance which, in the mammalian spermatozoön, forms a thin covering over the head and the proximal part of the tail. But there is another constituent which the recent studies have shown to be constant in all spermatozoa of all species, from the lowest to the highest groups. These are the chondriosomes. The same studies have shown also that the chondriosomes can assume in the ripe spermatozoön a shape and location which vary greatly in the different species. One used to say that the chondriosomes form a sheath around the "middle-piece" of the spermatozoön. This is a very loose, and in many cases an entirely incorrect statement. Loose, since under the term "middle-piece" we designate parts that have nothing in common: there is no homology whatsoever between the so-called "middle-piece" of the spermatozoön of an echinoderm, of a selachian (or an amphibian) and of a mammal. In many cases it is also an incorrect statement. In a number of higher vertebrates, especially in mammals, the chondriosomes actually form a sheath around what is customarily called the "middle-piece" of the spermatozoön; at the same time, the shape of that sheath varies a good deal, from isolated granules to discs or a spiral filament. In lower vertebrates (in many fishes) and in a great number of invertebrates (many molluscs, certain worms, etc.), the chondriosomes are represented by a small number of granules located on the posterior part of the head and surrounding the origin of the tail. A third type often met with, especially in insects, is represented by a long, thin sheath, which envelops the axile filament; this sheath is derived from the so-called "Nebenkern," which usually breaks in two, the pieces afterwards becoming elongated as the tail grows. But besides these three main types there exists also an infinity of other forms. Quoting at random I would mention the spermatozoa of *Ascaris*, *Phallusia*, *Ciona*, of many worms, crustacea, molluscs, etc. It is consequently impossible, in the presence of the ripe spermatozoön only, to decide what part of it is formed by the chondriosomes[1] and only a careful study of spermiogenesis can solve the problem. Indeed such a study should be undertaken every time one has to deal with atypical forms of spermatozoa as a preliminary to the study of fertilization.

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[1] This is a reason why I cannot, without further investigation, consider F. R. Lillie's observations on fertilization in *Nereis* as demonstrating that in that species the male chondriosomes do not penetrate into the egg.

The chondriosomes being a constant constituent of the spermatozoön, have we any clue as to their significance? Opinions differ on this point. Benda is responsible for a hypothesis according to which the chondriosomes are in some way connected with the property of motility. This hypothesis reappears occasionally, and in my opinion very unfortunately, since it is not supported by anything and is contradicted by a number of facts. Of course, one can well imagine how such an idea might be suggested by the appearance of the spiral filament of certain spermatozoa, just as a rather naïve theory of muscular contraction was once built upon the conception that the myofibrils were shaped like a spring. But I fail utterly to see how other forms of the chondriosomal sheath, such as the small granules existing in so many lower vertebrates and in many invertebrates, could suggest in any way a relation with the motility of the spermatozoön. Furthermore, Benda's hypothesis is in contradiction to Meves's experiments of merotomy on spermatozoa and to the presence of chondriosomes in those spermatozoa that make no active movements, such as the spermatozoa of decapods. The same facts disagree also with the idea expressed by Regaud, for whom "le chondriome du spermatozoide est moins un matériel héréditaire qu'une partie de la cellule jouant un rôle actuel de fixation et de concentration des substances ambiantes destinées à être consommées lors de la contraction du filament axile."

Another opinion was expressed by Koltzoff. This author considers the chondriosomes as playing the rôle "einer formbildenden Substanz." While this is apparently true for certain spermatozoa he studied, for decapods for instance, I would deny that Koltzoff's interpretation has any general value. On physical grounds it would seem impossible that bodies which are entirely enclosed in a cell, without coming in contact with its surface, could have anything to do with the form of that cell. To return to the type of spermatozoön so common among lower vertebrates and invertebrates, is it possible to imagine that these granules have anything to do with determining the form of the spermatozoön? The answer can only be a negative one.

Finally we come to the hypothesis according to which the chondriosomes represent the idioplasm contained in the protoplasm of the seminal cells. We must find out first what becomes of the male chondriosomes in the process of fertilization. Although one of the first studies of this process, that made on *Ascaris megalocephala* by van Beneden, showed that the whole spermatozoön may enter the egg, the great majority of biologists have accepted the dogma of the monopoly of the nucleus as carrier of the idioplasm. The reasons for that attitude are obvious. First, in most cases, only the nucleus of the spermatozoön was detected in the egg; further, investigators were quite naturally hypnotized by the beautiful karyokinetic figures which they saw formed in the fertilized egg at the expense of the pronuclei. Yet, after van Beneden, a number of other authors (Kostanecki, Nekrassof, Lams, etc., in invertebrates; Fick, Nicolas, Michaelis, Van der Stricht, etc., in vertebrates) have demonstrated that other parts of the spermatozoön are also carried into the egg. The hypothesis in which the chondriosomes represent an idioplasmic substance has given a renewed interest to these observations which had been considered as exceptional cases, and recent observations, made under the impulse of the same hypothesis, are likely to lead us to a reconsideration of our conception of the morphology of fertilization. I need only mention in this connection the case of the sea-urchin. It was generally accepted that only the nucleus and the so-called "middle-piece" penetrate into the egg and that the tail is left without. Recent observations, however, made with special care and a more refined technique, have shown that the tail also enters. Let us not forget, however, that the crucial point to determine is not whether the tail, but whether the chondriosomes are carried into the egg. As I pointed out when speaking of the structure of the spermatozoön, these questions have no connection. One can readily conceive of a type of spermatozoön in which only the head would penetrate, and yet the chondriosomes would enter the egg at the same time. Such a type exists, as a matter of fact, in a number of invertebrates, for instance the ascidians *Phallusia* and *Ciona*.[1]

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[1] In these two species, however, the tail also is found in the egg.

We have observations, consequently, showing that the tail of the spermatozoön, or part of it, penetrates into the egg. We come now to the cases where the chondriosomes have actually been found in the egg. Most of these cases have been described recently under the impulse of the chondriosomal theory, and, considering the short time intervening since the publication of that theory, their number is already quite impressive. The actual penetration of chondriosomes into the egg was seen long ago, however, by L. and R. Zoja in *Ascaris*, and later by Vander Stricht in the bat. To these observations have been added, during the last seven years, a number of others: on the sea-urchin, *Phallusia*, *Mytilus* and *Filaria* (Meves) and on *Ciona* (Duesberg). Thus we already have observations on a number of invertebrates (worms, ascidians, echinoderms and molluscs) and vertebrates (mammals and amphibians—for there is no doubt from the observations of Fick and Michaelis that in *Triton* and *Axolotl* also the male chondriosomes are carried into the egg).

While things would thus appear to be in perfect agreement with the theory according to which the chondriosomes are idioplasm, a difficulty, and a very serious one indeed, has arisen. First, Vander Stricht and his pupils found in mammals that the chondriosomal part of the spermatozoön (in the bat, the spiral filament) passes unchanged into one of the first two blastomeres. Similarly in the sea-urchin, the chondriosomal middle-piece is found in one of the daughter-cells after the first division (Meves). So far the difficulty could be met, for there is some reason to believe that the two first blastomeres of the mammalian egg are not equivalent, one of them possibly forming the trophoblast only, the other the embryo, and a similar hypothesis could be formulated concerning the sea-urchin, since the adult is formed of parts only of the original embryo. Meves supposed that the male chondriosomal substance would go over into these cells which build up the definite animal. His further investigations on the fate of the middle-piece, up to 32 blastomeres, show that its fate is variable and that certainly not all cells of the young sea-urchin will receive male chondriosomal substance.

Here lies the real difficulty, and its weight seems overwhelming. I would however warn against too hasty conclusions. A number of considerations: the constancy of the chondriosomes in the spermatozoön, the lack of a satisfactory hypothesis as to their significance[1] and the fact that in a number of adequately investigated cases they have been found to penetrate into the egg, render, in my opinion, further research necessary before a decision can be reached. The study of fertilization is indeed a difficult one, especially the investigation of such minute constituents of the spermatozoön amid the huge mass of the egg, but it is of such fundamental importance that the investigator will find in it his reward.

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[1] Since the middle piece of the spermatozoön does not change its shape nor its volume in the egg of the sea-urchin, it can apparently not be considered as having the value of a chemical factor, such as postulated by Loeb in his theory of fertilization.

THE EFFECT OF SOME FOOD HORMONES AND GLANDULAR PRODUCTS ON THE RATE OF GROWTH OF PARAMECIUM CAUDATUM.[1]

MARY H. CHAMBERS.

The animal body is adjusted to live upon plant or animal tissue. It is generally accepted now that the adequate diet must contain more than protein, carbohydrate, and fat, namely, an accessory factor or vitamin. Recent investigations upon dietaries of infants have shown the importance of some of the accessory factors. Hopkins (1912) added yeast to a diet of purified food stuffs and found that it accelerated the growth of rats. The same result was obtained later by Funk and Macallum (1915). More recently Osborne and Mendel (1917) have shown that the addition of yeast to the ration not only causes an increase in rate of growth of rats, but it acts as a stimulus to the appetite, and when removed from the diet growth ceases and decline ultimately follows. As yeast supplies a definite substance for growth so also potato juice acts as a corrective in cases of malnutrition. With these results in mind a series of experiments was undertaken to determine whether or not extracts of yeast and potato juice added to the diet of *Paramecium* produced any marked effect on the rate of division which according to Calkins (1902) is the index to vitality. The investigations were carried on under the direction of Dr. Florence Peebles, to whom the writer is indebted for suggestions and assistance.

A first set of experiments was made upon a pure line of *Paramecia* which had been maintained for several months on a diet of malted milk.

The solutions of yeast and potato which were employed were 1 per cent., and were made with distilled water. There were two methods of preparation with each. According to the first method the compressed yeast cake was merely dissolved in water, in the second, the yeast was first ground in order to allow the contents of the cells to enter the solution. One potato solution was boiled and strained, the other was strained raw. The controls were maintained in .2 per cent. solution malted milk. It was customary to feed the control and experimental lines an equal quantity of freshly made solutions every other day. The same food pipette was always used. Isolating pipettes were sterilized in boiling water each time used to avoid contamination in change of food and to kill any *Paramecia* that might have remained in the pipette. Such sterilization is not sufficient to destroy bacteria. All *Paramecia* were kept in depression slides in a moist chamber at constant temperature.

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[1] From the Biological Laboratory, Bryn Mawr College.

I. EXPERIMENTS WITH FOOD HORMONES.

The following tables show the number of divisions in several control and experimental lines. This experiment was carried on for twelve days and a record kept of each daily division. The table shows the data averaged in periods of six days each. Varying proportions of food substances were used. Although the total quantity of food was always the same in both lines, one set of experiments contained two drops of raw potato solution and one drop of the basic food substance—malted milk—with a control of three drops of malted milk;—and another—three drops of raw potato solution with a control of four drops malted milk.

TABLE I.

POTATO (RAW). Daily Division Average For Six Day Periods.

Period.	Control.	Potato, Two Drops.	Control.	Potato, Three Drops.
1	5.5	7.5	4	7
2	8	6	5.5	7.5
Total	13.5	13.5	9.5	14.5
Average	1.11	1.11	.78	1.2

With raw potato the average rate of growth in the weaker solution was practically the same as that of the control while the rate of division for the stronger extract exceeded that of the control.

TABLE II.

POTATO (BOILED). Daily Division Average For Six Day Periods.

Period.	Control.	Potato, Two Drops.	Control.	Potato, Three Drops.
1 2	6.5 6	4 8.5	7 6	7 5.5
Total	12.5	12.5	13	12.5
Average	1.03	1.03	1.08	1.03

It will be observed that when the potato extract was boiled the average rate of division was about the same for the weaker and stronger solutions.

A similar experiment was carried on at the same time with yeast. Two and three drops each of the whole and ground yeast were used.

TABLE III.

YEAST (WHOLE). Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Two Drops.	Control.	Yeast, Three Drops.
1 2	7 4	6 7	4.5 3.5	7 8.5
Total	11	13	8	15.5
Average	.91	1.08	.66	1.28

As this table shows, the individuals in the solution containing whole yeast divided more rapidly than the control.

TABLE IV.

YEAST (GROUND). Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Two Drops.	Control.	Yeast, Three Drops.
1 2	3 0	7.5 9	6 4.5	5 8.5
Total	3	16.5	10.5	13.5
Average	1.5	1.37	0.86	1.11

In this case a decided increase in the rate of division is shown when ground yeast is added to the diet.

A second set of experiments was made upon four races of *Paramecia* as follows: Race A had been in spring water in the laboratory for several months; race B came from a culture procured from the University of Pennsylvania; race C came from Powers and Powers, Nebraska; race D, was a wild race recently obtained from a pond in the neighborhood of the college laboratory.

A comparative study of pure lines from these four races was carried on with the addition of yeast both whole and ground to the food substance. Two drops each were used. Malted milk remained the basic food substance.

Table V.

A. Laboratory Culture in Spring Water. Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast. Ground.
1	16	11.5	9.5	11
2	10	10	9.5	13.5
Total	26	21.5	19	24.5
Average	2.6	1.78	1.41	2.03

These individuals had previously undergone a period of starvation living in the laboratory under adverse conditions. Here the malted milk alone seemed more favorable to growth without the addition of whole yeast. It will be observed, however, that when ground yeast was added that there was a distinctly marked increase in the division rate.

Table VI.

B. Laboratory Culture in Hay Infusion. Daily Division Average For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast. Ground.
1	12.5	10	10.5	10
2	13	10.5	8.5	14
Total	25.5	20.5	19	24
Average	2.11	1.7	1.6	2

The same result may be seen as in Table V., where the *Paramecia* had been in hay infusion.

TABLE VII.

C. MIXED CULTURE, POWERS AND POWERS. Daily Division Averaged for Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	6.5	7	10	10.5
2	7	7	6	3
Total	13.5	14	16	13.5
Average	1.11	1.16	1.33	1.11

This was a rich culture containing many infusoria, and a high percentage of bacteria. In these individuals the rate showed little increase above the average.

TABLE VIII.

D. WILD RACE. Daily Division Averaged For Six Day Periods.

Period.	Control.	Yeast, Whole.	Control.	Yeast, Ground.
1	9	5	11	7.5
2	12	2.5	8	1.5
Total	21	7.5	19	9
Average	1.75	.61	1.14	.75

The wild race of *Paramecia* had to undergo the greatest change. It thrived as the others on the rich diet of malted milk, but the experimental lines decreased exceedingly in division rate.

In the comparative study of these races it must be concluded that, (1) yeast stimulates growth as indicated by the rate of division, and (2) ground yeast is more favorable as food than whole yeast.

The physiological condition of the individual must be taken into consideration. For example in races A and B there was a higher rate of division than in races C and D. The latter ones had been in rich cultures before they were subjected to the experiment. Such a series also reveals the great variation in the rate for different individuals under the control conditions. For example, in Table V the variation of the control is 4.5, in Table VI., 3.2, in Table VII., 1.3 and Table VIII., 0.34. Although every effort was made to keep the conditions the same there is evidence of great variation.

In conclusion, the experiments performed with food hormones indicate (1) that potato extract will serve as a food but not as a growth accelerator, (2) that yeast extract serves as a food and stimulates growth, and (3) that the physiological condition of the individual plays a large part in determining the rate of division under experimental conditions. Results indicate that when food supply has been reduced a change of diet causes rapid increase in rate. Where food has been plentiful the rate of division has not been greatly increased.

2. Experiments with Glandular Diet.

Shumway (1917) has recently made a series of experiments with gland extracts. His controls were kept in a suspension of hay infusion. When adrenalin and pituitary extract were added these lines divided more than twice as rapidly as the control and with very little difference between them. In another experiment he determined to try what he termed a more crucial test,—*i. e.*, keeping the experimental lines in gland suspension alone without the basic food substance of hay infusion. Both lines died after a few days treatment.

For the following experiments a wild race of *Paramecia* was collected in January, 1918. Individuals descended from one original animal were put in hay infusion. On February 9, these *Paramecia* as control lines, were isolated in depression slides in a few drops of hay infusion and put in the moist chamber. Within twenty-four hours the sister *Paramecia* were isolated and the experimental lines established. The individuals were fed on solutions made from the tablets prepared in the laboratory of Armour & Co. The pituitary tablets were made from the desiccated gland of an ox and suprarenal from the desiccated gland of a sheep. The strength of the solution of the pituitary gland was .35 per cent., that of the suprarenal .36 per cent., being slightly stronger.

The experiments as recorded in Table IX. and Table X. were carried on for five weeks. With the pituitary solutions three proportions of food were used—first, control line two drops of hay infusion, experimental line one drop of hay infusion and one drop pituitary, second, control line three drops of hay infusion, experimental line one drop of hay infusion and two drops of pituitary; third, control line four drops of hay infusion,—experimental line one drop of hay infusion and three drops of pituitary.

Table IX.

Pituitary and Hay Infusion. Daily Division Average For Six-Day Periods.

Period.	Control.	Pituitary, One Drop.	Control.	Pituitary, Two Drops.	Control.	Pituitary, Three Drops.
1	.5	.66	.83	1.08	.5	.83
2	.5	1.0	.5	1.2	.92	1.0
3	.0	1.0	.83	.5	.66	1.3
4	.33	.75	.83	.5	1.2	.0
5	.5	1.0	.0	.25	.0	.33
Total	1.83	4.41	2.99	3.53	3.28	3.46
Average	.36	.88	.59	.65	.65	.69

There is an increase in division rate of experimental over control lines, but the amount of increase diminishes with an increasing amount of pituitary as food.

With the suprarenal two proportions of food were used;—first, control line two drops of hay infusion, experimental line one drop hay infusion, and one drop of suprarenal; second, control line three drops of hay infusion, experimental line one drop of hay infusion and two drops of suprarenal.

Table X.

Suprarenal and Hay Infusion. Daily Division Averaged For Six-Day Periods.

Period.	Control.	Suprarenal, One Drop	Control.	Suprarenal, Two Drops.
1	.8	1.0	1.5	1.1
2	.75	1.6	.66	1.1
3	1	.58	.0	1.0
4	1	.83	.66	1.2
5	.5	.66	.25	.41
Total	4.05	4.67	3.07	4.81
Average	.72	.943	.76	.96

These results are more constant than those obtained by feeding pituitary solution. There is a slight but decided increase over control lines.

The control of the experimental line containing two drops of suprarenal died and it was necessary to supply from stock which was kept in hay infusion.

A series of experiments was tried with mixtures of pituitary and suprarenal solutions in the basic food substance. The results indicate that the rate of division is greatly reduced. Sometimes the *Paramecia* would exist for seven days without dividing.

At this point in the work the control culture medium was changed from hay infusion to .2 per cent. malted milk. Another pedigreed race was started which had been in malted milk for several months. The solutions used were in two proportions—first, control line two drops of malted milk, experimental line one drop of malted milk, one drop of pituitary; second, control line three drops of malted milk, experimental line one drop of malted milk, and two drops of pituitary.

TABLE XI.

PITUITARY AND MALTED MILK SOLUTIONS. Daily Division Averaged for Nine Days.

Period.	Control.	Suprarenal, One Drop.	Control.	Suprarenal, Two Drops.
1	.89	0	1.1	0

In both experimental lines the *Paramecia* died after swimming about normally for five days without dividing. In no way can these results be correlated with the previous similar experiment where the basic food was hay infusion.

Solutions of suprarenal were the same as those above for pituitary.

TABLE XII.

SUPRARENAL AND MALTED MILK SOLUTION. Daily Division Average for Nine Days.

Period.	Control.	Suprarenal, One Drop.	Control.	Suprarenal, Two Drops.
1	.83	1	.77	1.1

There is an increase in division rate when Paramecia are fed suprarenal solution whether the basic fluid is hay infusion or malted milk.

CONCLUSIONS.

As a food hormone, potato extract apparently has little effect on the division rate of *Paramecium.* The influence of yeast is evident as exemplified in Tables III. and IV., *i. e.*, it increases the division rate.

In the comparative study of the four races the influence of yeast is not evident. Both control and experimental lines respond with increased division rate if the Paramecia have previously been in a starved condition. If food has been plentiful the rich diet of malted milk and yeast seems to have little effect.

Contrasting results were obtained with pituitary solution added to the hay infusion and malted milk basic substances. Suprarenal extract added to the basic fluid in which the *Paramecia* lived, caused an increase in the rate of division.

This experiment is to be considered as an indicator only. It was undertaken with the point in mind that if these solutions influenced the division rate of *Paramecia* a more extensive work would follow, *i. e.*, the bacterial content would be taken into consideration. It has been a generally accepted fact that the normal diet of *Paramecium* is composed of various kinds of bacteria and that in their absence growth does not take place, and even maintenance is impossible. In 1908 Jennings said, "To make the conditions of existence the same, it is not sufficient to attend merely to the basic fluid, the bacteria must also be the same."

Hargitt and Fray (1917) elaborated on previous methods of determining the bacterial content and they proved that *Paramecium* can live in media which have undergone sterilization provided at least one sort of bacteria remains in the culture.

LITERATURE.

Buddington, R. A.

'15 Division Rate in Ciliate Protozoa as Influenced by Thyroid Constituents. BIOL. BULL., Vol. 28, p. 304.

Calkins, Gary N.

'02 Studies on the Life History of Protozoa. Arch. f. Ent. Mech., Vol. 15, pp. 139–186.

Funk, C., and Macallum, A. B.

'15 The Action of Yeast Fractions on the Growth of Rats. Jour. Biol. Chem., Vol. 23, p. 63.

Hargitt, Geo. T., and Fray, W. T.

'17 The Growth of Paramecium in Pure Cultures of Bacteria. Jour. Exp. Zoöl., Vol. 22, no. 2, p. 421.

Hopkins, F. G.

'12 Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Dietaries. Jour. of Phys., Vol. 44, p. 425.

Jennings, H. S.

'08 Hereditary Variations and Evolution in Protozoa. Proc. Amer. Philos. Soc., Vol. 47, pp. 393–546.

Macallum, E. V., and Kennedy, C.

'16 The Dietary Factors Operating in the Production of Polyneuritis. Jour. of Biol. Chem., Vol. 24.

Osborne, F. B., and Mendel, L. B.

'17 The Rôle of Vitamines in the Diet. Jour. of Biol. Chem., Vol. 31.

Shumway, Waldo.

'17 The Effect of a Thyroid Diet upon Paramecia. Jour. of Exp. Zoöl., Vol. 22, no. 3.

A FRESHWATER ANAËROBIC CILIATE.

CHANCEY JUDAY.

Investigations on a considerable number of Wisconsin lakes have shown that, in many of them, more or less of the lower stratum, or hypolimnion, and the muddy ooze at the bottom possess no free oxygen for a certain time during the summer stagnation period (Birge and Juday, '11). This time varies from three or four weeks in some lakes to as many months in others. The dissolved oxygen disappears gradually from this stratum as the season advances and when it reaches a minimal amount the various organisms occupying this region respond to the change either by migrating or by adapting themselves to the new conditions.

When the minimum is reached for plankton crustacea they rise to a higher level where oxygen is more abundant and when this gas becomes too scarce for some of the bottom dwelling insect larvæ they migrate to the shallower water. The majority of the forms, however, are able to remain here even in the absence of free oxygen. To this latter group belong representatives of at least seventeen genera of protozoa, including rhizopods, flagellates and ciliates, but chiefly ciliates, and a number of higher forms, such as insect larvæ, an ostracod, worms, and a bivalve mollusk. These forms appear to thrive as well in water which contains no free oxygen as in water which is well aërated, thus being facultative anaërobes (Juday, '08).

While making a study of the centrifuge plankton of Lake Mendota a ciliate was found in the lower stratum of water which appears to have gone beyond the facultative stage so that it has become substantially a true anaërobe. This ciliate agrees very closely in shape and structure with Fig. 126, Plate X., of Conn's "Protozoa of the Fresh Waters of Connecticut," which this author "with hesitation" places provisionally in the genus *Enchelys*. It has uniform ciliation, a rather pointed, obliquely truncate anterior end, and a rounded posterior extremity. Frequently the anterior third of the organism appears to be filled with minute granules which give this portion a somewhat darker color. The maximum length ranges from 35 μ to 40 μ and the greatest width from 15 μ to 17 μ.

This ciliate was found each season from 1914 to 1917 inclusive, but it was most abundant in the former year and scarcest in the latter. The accompanying table shows its vertical distribution in four sets of observations which are typical of the entire series. This table also shows the relation of the organism to temperature, dissolved oxygen, and free carbon dioxide. The observations were made at a regular station where the lake has a depth of 23.5 meters.

Table I.

This Table Shows the Vertical Distribution of the Ciliate and its Relation to Temperature, Dissolved Oxygen and Free Carbon Dioxide.

Date.	Depth in Meters.	Number of Organisms per Liter.	Temperature, Degrees C.	Oxygen, c.c. per Liter.	Carbon Dioxide, c.c. per Liter.
October 10, 1914..........	14	0	17.4	1.67	2.50
	15	90,700	17.0	0.14	2.83
	16	58,900	16.3	0.00	3.92
	17	27,200	14.5	0.00	4.58
	18	0	13.8	0.00	5.14
October 21, 1914..........	17	0	15.2	0.60	3.53
	18	68,000	15.1	0.00	5.50
	19	68,000	14.6	0.00	7.92
	20	22,700	14.2	0.00	9.00
	21	9,100	13.3	0.00	9.24
	22.5	9,100	12.6	0.00	11.00
September 22, 1915.......	15	0	16.9	0.40	2.40
	16	27,200	15.9	0.00	5.67
	17	27,200	15.4	0.00	
	18	6,800	15.2	0.00	7.14
	20	0		0.00	
September 20, 1916.......	15	0	17.0	3.08	3.54
	16	13,600	14.8	0.00	7.33
	17	6,800	14.4	0.00	8.08
	18	6,800	14.0	0.00	8.84
	20	0	13.8	0.00	9.45

In 1914 regular observations were not begun until the first of September and this ciliate was first noted on September 16. The number rose to a maximum of 95,200 individuals per liter of water at a depth of 16 meters on September 29. From the latter date until October 21 the organism was abundant, but by October 23 the number had decreased 70 per cent., and by October

27 it had entirely disappeared. The autumnal overturn took place between the last two dates, with the consequent aëration of the water at all depths, and this event was followed by the prompt disappearance of the ciliate.

In 1915 this organism was found in small numbers in four sets of observations between July 12 and July 22. The stratum of water which it occupied at this time contained from 0.10 c.c. to 0.40 c.c. of dissolved oxygen per liter. Following the latter date it was not found again until August 19 and August 23 when a small number was noted in a stratum of water which contained no dissolved oxygen. It appeared regularly in catches made during September, the largest number being found during the latter part of this month. It disappeared between September 29 and October 6, the autumnal overturn and aëration of the lower water having taken place in the meantime.

In 1916 this ciliate was noted twice in July, but it did not appear again until September 18, when it was obtained during the following ten days. In 1917 it was found only in September and then only in small numbers.

This protozoan showed a downward movement each autumn which was correlated with the descent of the thermocline or mesolimnion, the organism keeping just below the stratum of aërated water. Thus in the table above it will be noted that the stratum occupied on October 21, 1914, was three meters deeper than that of October 10. This descent continued until the bottom was reached and then the ciliate disappeared promptly, usually within two days, after the bottom water became aërated. In the laboratory it lived in water that had been partially aërated only for a period of about twenty-four hours.

As indicated in the table there did not seem to be any correlation between the vertical distribution of this ciliate and the temperature of the water or the amount of free carbon dioxide, but there was a definite correlation with the lack of dissolved oxygen, the stratum occupied having at the most only a minimal amount of this gas and most frequently none at all. This fact and the further fact that it disappeared so promptly when the water became well aërated seem to warrant the conclusion that it is substantially an anaërobic ciliate.

LITERATURE.

Birge, E. A., and Juday, C.

'11 The Inland Lakes of Wisconsin. The Dissolved Gases and their Biological Significance. Bull. XXII., Wis. Geol. & Nat. Hist. Survey, pp. 20 and 259.

Conn, H. W.

'05 A Preliminary Report on the Protozoa of the Fresh Waters of Connecticut. Conn. Pub. Doc. No. 47, Bul. No. 2, Geol. & Nat. Hist. Survey, pp. 69, pls. 34.

Imel, H. G.

'15 Some Preliminary Observations on the Oxygenless Region of Center Lake, Kosciusko County, Indiana. Proc. Ind. Acad. Sci. (1916), pp. 345–356.

Juday, C.

'08 Some Aquatic Invertebrates that Live under Anaërobic Conditions. Trans. Wis. Acad. Sci., Arts and Let., Vol. XVI., Part 1, pp. 10–16.

NOTE ON ANIMAL DISTRIBUTION FOLLOWING A HARD WINTER.

W. C. ALLEE.

LAKE FOREST COLLEGE.

For the past six summers I have been studying the distribution of littoral invertebrates near Woods Hole, Mass., in connection with a teaching appointment in the invertebrate course in the Marine Biological Laboratory. The work has been largely done in connection with the class trips which give a good opportunity for general collecting in that each of the six or seven instructors has from six to eight students working with him in the field. As a result of the class collecting during this period, there are over 190 species of invertebrates listed in the class catalogue that have been taken alive on student excursions.

Something of the thoroughness of the collecting may be seen from the fact that this work has added two new species to the faunal catalogue of this well-studied region: In 1915 Dr. T. S. Painter took a living specimen of *Arca ponderosa* (Say) in dredging off Naushon in Vineyard Sound. The animal was taken on the sand dollar grounds off Tarpaulin Cove in about six fathoms of water. Regarding this species the Biological Survey[1] says: "Verrill has expressed doubts as to whether *Arca ponderosa* lives in this region since no living specimen has been taken north of Cape Hatteras. Mr. G. M. Gray likewise reports that he has never taken this species alive. Dr. Dall informs us, however, that the National Museum contains a fresh valve, retaining the epidermis, taken in Vineyard Sound in 1870; and Mr. C. M. Johnson reports that he has found more than one shell of this species still bearing evident traces of the hinge ligament and epidermis, on a beach near Chatham Light." The extensive dredgings of the survey yielded only a few shells. The range is usually given as extending from Provincetown to Yucatan.

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[1] Biological Survey of the Waters of Woods Hole and Vicinity by Francis B. Sumner, Raymond C. Osborn, and Leon J. Cole. Bull. Bureau of Fisheries, Vol. 31. Two parts.

Miss Eleanor Bach in 1916 found a specimen of *Halicystus auricula* (Clark) attached to eel grass in low water near the old Vineyard Haven wharf where it had apparently been washed up with algæ. This is a northward ranging species with a range from Cape Cod to Greenland.

A slight amount of preliminary collecting in the latter part of June, 1918, showed that the fauna was noticeably changed from the preceding year and that the change was decidedly greater than the usual yearly fluctuation. The preceding winter had been unusually severe and this offered an hypothesis for the cause of the unusual changes in the abundance of some animals.

The water temperatures have been taken for a number of

Table I.

Showing the Temperatures at Noon off the U. S. Fish Commission Wharf at Woods Hole, for the Four Winter Months of 1916–17 and 1917–18.

1916–17.					1917–18.			
Dec.	Jan.	Feb.	Mar.		Dec.	Jan.	Feb.	Mar.
46.5	32	33	32.5	1	41	29.5	30	32
45	33.5	32	32.5	2	40.5	29.5	30	32
44.5	34.5	30	32	3	39	30	30	32
45	34.5	30	32	4	39.5	29.5	30	31.5
46.5	34.5	30	32	5	39.5	30	30	32
46	35.5	30	32	6	39	30	30	32.5
44.5	35.5	30.5	32.5	7	38.5	31	30	32
44.5	35	31	33.5	8	37	30.5	30	31.5
44.5	36	31.5	34	9	38	30	30	31.5
44	36.5	31	33.5	10	36	30.5	30	32
43	36	31.5	33	11	35	31	30	31.5
44	33	30	35.5	12	34.5	31	30	31.5
42.5	33.5	30	33.5	13	34	30	31	32
42	35	30	34	14	34.5	30	31	31
41	33.5	30	34.5	15	33	30	31.5	31.5
39.5	33.5	31	34.5	16	33	30.5	31.5	30.5
39.5	32.5	31	34.5	17	33	30	31.5	30.5
38	33	31	35.5	18	33.5	30	31	31.5
37	32.5	31	34	19	33	30.5	31.5	32.5
36	32.5	31.5	34	20	33.5	30	32	32.5
37	32.5	31	34	21	34.5	30	31	33
37	33	31	34.5	22	34.5	30.5	31	32.5
37	32.5	30.5	34	23	33	30	31	34
36	32.5	31.5	35.5	24	33	30	31	33
36.5	32.5	31.5	36	25	34.5	30.5	31	34
35	32	32	37.5	26	33	30.5	32	34
35	31	32	37.5	27	32.5	30.5	31	34.5
36.5	31	31	38.5	28	32.5	30.5	32	34.5
35	31.5		38	29	31	30		36
34	33		38	30	29.5	30		36
32.5	32.5		39	31	29.5	28		36.5

years off the wharf of the U. S. Fish Commission at Woods Hole. The manuscript notes of the daily readings for the winters of 1916–17 and 1917–18 were made accessible through the kindness of Superintendent W. H. Thomas. The noon temperatures for the months of December, January, February and March of these years are given in Table I. From December 29, 1917, to March 19, 1918, a period of 80 days, the noon temperature did not rise above freezing, except on March 6, when it reached 32.5° F.

These temperatures are summarized in Table II., together with a similar summary for the period of 1902–06 taken from the Biological Survey. It will be noted that the mean temperature for each of the months considered is less than for the corresponding month of the preceding year or of the five-year interval; that February, the coldest month, is only slightly below that of the other years, but that the other months are all decidedly lower and that the cold weather continued practically through March.

TABLE II.

Showing Mean, Maximum and Minimum Noon Temperatures off the Fish Commission Wharf at Woods Hole, Mass.

Period.	December.	January.	February.	March.
1902–1906. Mean	37.2	32.3	31.0	35.64
Minimum	31.5	28.0[1]	28.0[1]	29.5
Maximum	47.5	39.5	37	44
1916–1917. Mean	39.9	33.4	30.9	34.3
Minimum	32.5	31	30	32
Maximum	46.5	36.5	33	39
1917–1918. Mean	35.1	30.1	30.75	32.7
Minimum	29.5	28[1]	30	30.5
Maximum	46.5	31	32	36.5

[1] Inexact; below the freezing point of water.

The mean noon temperature for the 121 days listed, in the five-year period from 1902–06 was 34.4 degrees. In 1916–17 there was a mean temperature of 35.5 degrees during this winter period, while for 1917–18 the mean temperature was only 33.3 degrees. This gives a temperature deficit of 1.1 degrees per day when compared with the longer period, and of 2.2 degrees when compared with the preceding winter.

The effect of an usually cold winter should be most marked upon animals near the northern limit of their geographical range. The result of an inquiry as to whether or not such animals were affected is shown in the list given as Table III. The list is made from the class list of invertebrates whose distribution, as shown in Pratt's Manual of Common Invertebrate Animals, is that of southern ranging animals. By "southern ranging" is meant that the known geographical range extends twice as far south as north of the point under consideration. The frequency data is based principally on my own experience in the field assisted by that of Mr. J. S. Kostir, of Ohio State University, who has collected over the same ground for the last two summers.

Table III.

A List of Species, that are South Ranging according to Pratt. Taken by the Invertebrate Class of the Marine Biological Laboratory. The Comments in the Last Column Give the Frequency Data as Compared with the Preceding Summer.

Species	Range	Frequency
Porifera:		
Cliona celata (Grant)	S. Ca.–Maine.	Fewer.
Chalina arbuscula (Ver.)	N. Ca.–Cape Cod.	Fewer.
Microciona prolifera (Ellis and Solander	S. Ca.[2]–Cape Cod.	Fewer.
Coelenterata:		
Pennaria tiarella (Ayres)	Fla.–Maine.	Fewer.
Schizotricha tenella (Ver.)	Beaufort,[1] N. C.–Marthas Vineyard.	No change.
Dactylometra quinquecirrha (Desor)	Tropics–Vineyard Sound.	Comparison unfair.
Sagartia luciæ (Ver.)	Florida[2]–Cape Cod.	Fewer.
Sagartia leucolena (Ver.)	N. Ca.—Cape Cod.	More.
Eleoactis producta (And.)	S. Ca.—Cape Cod.	Fewer.
Astrangia danæ (Ag.)	Fla.—Cape Cod.	Fewer.
Nemertini:		
Micruri leidyi (Ver.)	N. J.—Cape Ann.	Fewer.
Cerebratulus lacteus (Leidy)	Fla.—Maine.	Fewer.
Echinoderma:.		
Asterias forbesi (Desor)	Common south of Cape Cod Rare north to Maine.	More small.
Ophioderma brevispina (Say)	South from Cape Cod.	Fewer.
Arbacia punctulata (Gray)	Yucatan[2]—Cape Cod.	Fewer.
Thyone briareus (Lesueur)	South from Vineyard Sound.	No change.
Annelida:		
Sthenelais leidyi (Quatr.)	N. Ca.—Mass. Bay.	No change.

Podarka obscura (Ver.)	G. of Mexico—Cape Cod.	Fewer.
Nereis limbata (Ehlers)	S. Ca.—Maine.	No change.
Platynereis megalops (Ver.)	Beaufort, N. C.—Cape Cod.	Comparison unfair.
Diopatra cuprea (Bosc.)	S. Ca.—Cape Cod.	No change.
Lumbrinereis tenuis (Ver.)	Va.—Mass.	No change.
Arabella opalina (Ver.)	West Indies—Maine.	No change.
Glycera americana (Leidy)	S. Ca.—Cape Cod.	No change.
Scoloplos robustus (Ver.)	N. Ca.—Cape Cod.	No change.
Chætopterus pergamentaceus (Cuv.)	N. Ca.—Cape Cod.	No change.
Cirratulus grandis (Ver.)	Va.—Cape Cod.	No change.
Pista palmata (Ver.)	Va.[1]—Vineyard Sound.	Comparison unfair.
Amphritrite ornata (Leidy)	N. Ca.—Cape Cod.	No change.
Polycirrus eximeus Leidy)	N. Ca.—Cape Cod.	No change.
Pectinaria gouldi (Ver.)	N. Ca.—Maine.	No change.
Maldane urceolata (Leidy)	N. Ca.—Cape Cod.	No change.
Arenicola cristata (Stimp.)	Fla.—Cape Cod.	No change.
Trophonia affinis (Leidy)	N. J.—Vineyard Sound.	Comparison unfair.
Clymenella torquata (Leidy)	N. Ca.—Bay of Fundy.	No change.
Sabella micropthalmia (Ver.)	N. Ca.—Cape Cod.	Fewer.
Hydroides hexagonus (Bosc.)	Fla.—Cape Cod.	Fewer.
Sabellaria vulgaris (Ver.)	N. Ca.—Cape Cod.	Fewer.
Bryozoa:		
Bugula turrita (Desor)	N. Ca.—Casco Bay.	No change.
Schizoporella unicornis (Johnston)	S. Ca.[2]—Mass. Bay.	Fewer.
Arthropoda:		
Balanus eburneus (Gould)	W. Indies—Mass. Bay.	No change.
Orchestria agilis (Smith)	Ranges south to Florida.	No change
Talorchestria longicornis (Say)	N. J.—Cape Cod.	No change.
Haustorius arenarius (Slabber)	Ga.—Cape Cod.	No change.
Caprella geometrica (Say)	Va.—Cape Cod	Fewer.
Sphæroma quadridentatum (Say)	Fla.—Cape Cod.	Fewer.
Chiridotea cæca (Say)	Fla.—Nova Scotia.	Comparison unfair.
Palæmonetes vulgaris (Say)	Fla.—Mass.	No change.
Pagurus longicarpus (Say)	S. C.—Maine.	No change.
Pagurus pollicaris (Say)	Fla.—Maine.	No change.
Pagurus pollicaris (Say)	Fla.—Maine.	No change.
Hippa talpoida (Say)	Fla.[1]—Cape Cod.	Fewer adult.
Libinia emarginata (Leach)	Fla. Maine.	Fewer.
Libinia dubia (Milne-Ed.)	Fla.—Cape Cod.	More.
Panopeus sayi (Smith)	Fla.—Mass.	No change.
Callinectes sapidis (Rath.)	La.—Cape Cod.	Fewer.
Ovalipes ocelatus (Herbst)	G. of Mex.—Cape Cod.	Fewer.
Carcinides maenas (L.)	N. J. —Cape Cod.	Fewer.
Pinnotheres maculatus (Say)	S. C.—Cape Cod.	No change.

Uca pugilator (Bosc.)	Fla.—Cape Cod.	No change.
Limulus polyphemus (L.)	Fla.—Nova Scotia.	More.
Tanystylum orbiculare (Wilson)	Va.[1]—Martha's Vineyard.	Comparison unfair.
Mollusca:		
Chætopleura apiculata (Say)	Fla.—Cape Cod.	No change.
Melampus lineatus (Say)	Tex.—N. Eng.	No change.
Natica duplicata (Say)	Mex.—Mass. Bay.	No change.
Crepidula fornicata (L.)	S. Am.—Nova Scotia.	No change.
Crepidula convexa (Say)	Fla.—Nova Scotia.	No change.
Crepidula plana (Say)	Tex.—Maine.	No change.
Bittium alternatum (Say)	N. J.—Mass. Bay.	No change.
Cerithiopsis greeni (Adams)	Tex.—Mass. Bay.	No change.
Eupleura caudata (Say)	Fla.—Cape Cod.	Comparison unfair.
Urosalpinx cineros (Say)	Fla.—Mass. Bay.	No change.
Columbella lunata (Say)	Fla.—Mass. Bay.	No change.
Columbella avara (Say)	Fla.—Cape Cod.	Fewer.
Nassa trivitatta (Say)	Fla.—G. of St. Law.	No change.
Nassa obsoleta (Say)	W. Fla.—G. of St. Law.	No change.
Busycon carica (L.)	G. of Mex.—Cape Cod.	Fewer.
Busycon canaliculatum (L.)	G. of Mex.—Cape Cod.	Fewer.
Anomia ephippium (L.)	W. Ind.—Nova Scotia.	Fewer.
Arca pexata (Say)	Fla.—Maine.	Fewer.
Arca ponderosa (Say)	Fla.—Cape Cod.	Comparison unfair.
Arca transversa (Say)	Fla.—Cape Cod.	Fewer.
Ostrea virginica (Gmelin)	G. of Mex.—Mass.	Fewer.
Pecten irradians (Lam.)	Tex.—Cape Cod.	Fewer adult.
Tellina tenera (Say)	W. Fla.—G. of St. Law.	No change.
Cumingia tellinoides (Conrad)	Fla.—Cape Cod.	No change.
Venus mercenaria (L.)	Tex.—G. of St. Law.	No change.
Petricola pholadiformis (Lam.)	Tex.—G. of St. Law.	Fewer.
Laevicardium mortoni (Conrad)	W. Fla.—Nova Scotia.	Fewer.
Clidiophora trilineata (Say)	Tex.—Nova Scotia.	Comparison unfair.
Chordata:		
Dolichoglossus kowalevskyi (A. Agassiz)	Beaufort, N. C.—Mass. Bay.	No change.
Styela partita (Stimp.)	N. C.—Mass. Bay.	Fewer.
Molgula manhattanensis (DeKay)	N. C.—Casco Bay.	Fewer.
Perophora viridis (Ver.)	Bermuda[1]—Vineyard Sound.	Fewer.
Amaroucium pellucidum (Leidy)	N. C.—Vineyard Sound.	No change.
Amaroucium stellatum (Ver.)	N. C.—Cape Cod and north.	No change.

Summary: Fewer, 36; no change, 45; more, 5; unfair comparison, 9.

[1] Range taken from Biological Survey.

[2] Discussed in text following.

In order to check up on this data I asked Mr. G. M. Gray for a list of the forms that had proven difficult to obtain during the 1918 season. I did not ask him to refer to his records because only those animals that had been so difficult to obtain that they were well fixed in his mind were wanted. This group of animals with their distribution is given as Table IV. An inspection of the table will show that in each case the animals are southern ranging forms with Woods Hole near the northern limit of their range.

Table IV.

Listing the animals which Mr. G. M. Gray found to be especially difficult to obtain in the summer of 1918 as compared with his experience in other years.

Sagartia luciæ (Ver.)	Fla.—Mass. Bay.
Astrangia danæ (Ag.)	Fla.—Cape Cod.
Arbacia punctulata (Gray)	Yucutan—Cape Cod.
Hippa talpoida (Say)	Fla.—Cape Cod.
Callinectes sapidus (Rath)	La.—Cape Cod.
Styela partita (Stimp.)	N. C.—Mass. Bay.
Perophora viridis (Ver.)	Bermuda—Vineyard Sound.

Arbacia punctulata had been particularly abundant in 1917. Small specimens were taken from rocks at Kettle Cove, at the Buzzard Bay entrance to Northwest Gutter, near Quissett and at North Falmouth. In all these places they came up almost to the low tide limit. In the deeper water they had again appeared in numbers on what the collecting crew called "the old *Arbacia* grounds" off Nobska Light. In 1918 none were taken by the class except in dredging in Vineyard Sound off Tarpaulin Cove, and then only a few small individuals. In fact during all the summer, in place of the usual abundance of large specimens, Mr. Gray was able to furnish only relatively few small *Arbacia*.

Sagartia luciæ, usually the most abundant actinian in the Woods Hole region, was almost wanting at the beginning of the summer in 1918. In only one place visited throughout the summer was this small anemone present in anything like its usual abundance. This was at Kettle Cove, where in the protected tide pools, the rocks were covered in about their normal fashion. On the exposed parts of Kettle Cove, as everywhere else, the rocks were practically bare. Pratt gives the distribution of this species as Long Island Sound to Massachusetts Bay and further north. The Biological Survey says that it first appeared in New Haven in 1892 and at Woods Hole in 1898. Professor Verrill believed that this *Sagartia* was introduced near New Haven on oysters from the south. Professor Parker states that it is a southern ranging species.

One more particular instance of a marked decrease in abundance was that of the red sponge, *Microcione prolifera*. In the early part of the season this sponge was almost absent from the wharf piles. Later they became more abundant.

In a number of instances the cold weather killed off the animals living in shallow water and left only those in deeper water. This tendency has already been mentioned in the case of *Arbacia*. It was also marked with the encrusting bryozoa as, *Schizoporella unicornis*.

In Table III. the data concerning *Platynereis* is listed as being insufficient to furnish a fair comparison. As a matter of fact none were taken this year but usually the number taken in the class collecting has been small and it is possible that we overlooked them this year. However, Dr. E. E. Just, who has been collecting swarming annelids for some years, reported that up to August 1 he had taken no *Platynereis* in his night collections although usually they are abundant by that time. *Autolytus cornutus* (A. Agassiz) was also lacking in his collections at that date although normally present. Unlike the other species this annelid is northerly ranging. It is found from New Jersey to the Bay of Fundy. Dr. Just also reported that the cold spring had affected the periodicity[1] of the early runs of *Nereis limbata* in a way wholly comparable with other late springs.

It is to be expected that the negative effect of a cold winter would be more noticeable than its positive effect. However, two northerly ranging species were present in decidedly increased numbers in the collecting. The more noticeable of these was the sponge, *Leucosolenia botryoides* (Bow.). Pratt gives its range as from Martha's Vineyard to the Gulf of St. Lawrence. Usually we have taken it to some extent in the class collecting on wharf pilings, but this year it was very abundant there and was found in some abundance on rocks, such as those at the Buzzard Bay entrance to Northwest Gutter.

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[1] Lillie, F. R., and Just, E. E., 1913, "Breeding Habits of the Heteronereis. Form of *Nereis limbata* at Woods Hole, Mass.," BIOL. BULL., 24, pp. 147–169.

The blood starfish, *Henricia sanguinolenta* (O. F. Müller), distributed from Cape Hatteras to Greenland and reported as common north of Cape Cod, was decidedly more abundant this year than usual.

The effects of the unusually cold winter on the abundance of the animal life may be due to one or all of the following factors:

1. To the temperature deficit, regardless of the duration of the cold weather. In this case the deficit amounted to 133 degrees when the winter months were compared with those of the five year period of 1902–06.

2. To the long-continued cold weather (practically 80 days at or below freezing).

3. To the fact that the cold weather began relatively early, or that it lasted relatively late, without regard to the temperature in the mid-winter interval.

Of these possibilities, I am inclined to regard the second and third as furnishing the cause for the observed change on the animals, with the fact that the cold weather lasted well through March as the most important factor. This is supported by the February temperatures, which were practically the same for the three periods listed, so the temperature for this month could have made little difference, and by general experience that a "late spring" gives some approach to the conditions found this summer.

A SIMPLE METHOD FOR MEASURING CARBON DIOXIDE PRODUCED BY SMALL ORGANISMS.[1]

E. J. LUND.

The suitable methods available at the present time for measuring the carbon dioxide produced by single cells such as eggs and small organisms weighing not over one or two grams are limited in number and usually only suitable for special types of material. Among the best methods for measuring or comparing small quantities of carbon dioxide produced by small organisms are those by Thunberg ('05) as modified by Winterstein ('13), and Krogh ('16). These are in turn based on the older and well known methods of Pettenkoffer and Petterson. Another method is that used by Warburg ('09) in his studies on sea urchin eggs and a more recent and apparently extremely sensitive method for the detection and estimation of very small quantities of CO_2 is that of Tashiro ('17). All of these methods may have their own advantages, depending upon the material and the nature of the problem. However, if many separate determinations of CO_2 are to be made at the same time, the apparatus must be duplicated or if not, the complexity of the procedure is increased greatly and thus becomes practically impossible.

In attempting to devise some simple method for measuring at the same time the small amounts of CO_2 produced by separate samples of *Paramecium*, or small invertebrates weighing one gram or less, the following procedure was adopted. Fig. 1 and the photograph, Fig. 2, show the arrangement. Two bottles of about two liters capacity, one containing $n/100$ HCl and the other a solution of $Ba(OH)_2$ of any concentration between $n/75$ and $n/150$, are provided with soda lime tubes and burettes. The solutions should be made up with CO_2 free distilled water as described in Treadwell and Hall ('15). A bottle of about 100 c.c. capacity, has a large glass stopper which fits the bottle very accurately and from which is suspended a small dish by means of the hook and wire. The stender dish, Fig. 1, *d*, may either be suspended as shown or held rigidly by an appropriately shaped clamp of wire fixed to the stopper with cement.

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[1] From the Department of Animal Biology, University of Minnesota.

The procedure in brief is as follows: One to five cubic centimeters of a suspension of *Paramecium* which have previously been gradually transferred to pure tap water from native hay infusion are placed in the dish, Fig. 1, *d*. Ten or fifteen cubic centimeters of the $n/100$ $Ba(OH)_2$ solution are run into the bottle through a small hole in a cardboard disk which is placed over the mouth of the bottle shown in the photograph. The dish is then placed on the hook of the stopper and the bottle quickly stoppered as shown in Fig. 1. The ground glass stopper should be slightly greased and the bottle tightly stoppered. The CO_2 already in the air in the bottle and the CO_2 given off from the organisms in the suspended dish diffuses into the $Ba(OH)_2$ solution and quickly forms the insoluble white precipitate of $BaCO_3$. This absorption continues until no more $Ba(OH)_2$ remains if sufficient CO_2 is produced to neutralize all the free base, for the $BaCO_3$ is practically insoluble and hence the reaction goes practically to completion. At the end of the desired period, the length of which is determined by the rate of CO_2 production by the organisms and conditions of the experiment, the stopper is quickly removed and the bottle is again covered with

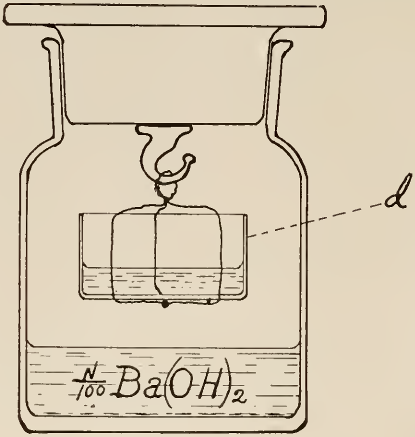

FIG. 1.

the cardboard disk. A drop of phenolphthalein is added through the hole in the cardboard disk and then the $Ba(OH)_2$ is rapidly titrated with a weak solution of HCl of known and appropriate concentration, say $n/100$. As control a similar bottle is set up in *exactly* the same way but *without* the animals. The $Ba(OH)_2$ is titrated at the end of the experiment, that is, at the same time as the free $Ba(OH)_2$ in the experimental bottle. The difference in the amounts of HCl necessary to neutralize the remaining free base in the bottles is equivalent to the quantity of CO_2 produced by the organisms during the period of the experiment. This description explains in brief the principle and procedure of the method, but the following sources of variation and error suggest themselves and will be considered in the same order as follows:

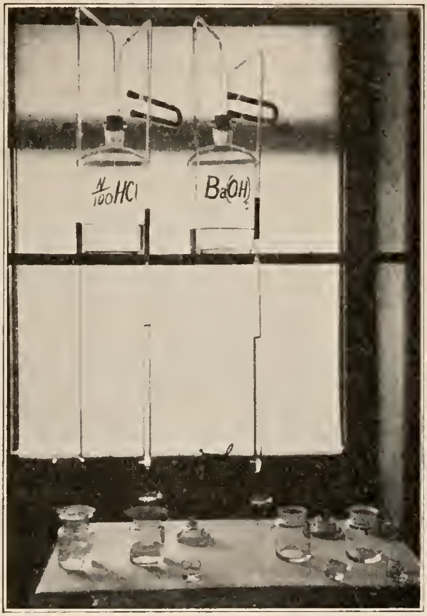

FIG. 2.

1. Temporary exposure to the CO_2 in the air of the open bottle containing the $Ba(OH)_2$ may result in absorption of varying amounts of CO_2 in different individual bottles. This variation

may be due to non-uniformity of manipulation and different CO_2 content of the air in the room. The greatest source of error comes from the CO_2 in the expired air of the experimenter and from burning flames in the room.

2. If the organisms are in water in the dish, Fig. 1, *d*, then the CO_2 dissolved in this water either as dissolved free CO_2 or possibly partly in the form of bicarbonate, may vary unless precautions are taken to control this source of variation of CO_2.

3. Another possible source of variation is the excretion of CO_2 by bacteria in the liquid in the dish, if this is for example a hay infusion containing *Paramecium* or from some other source depending upon the material experimented upon. This source of error is present and must be controlled in all other methods for CO_2 determination as well as in the one described here.

4. Finally perhaps the most important question is: how uniform, rapid and complete is the absorption of the CO_2 from the air in the bottle, by a $n/75$ to $n/150$ $Ba(OH)_2$ solution?

Repeated tests have shown that all of the above four sources of error can readily be either eliminated or controlled if the following precautions are taken.

The first source of error mentioned under number one above can be controlled and thus practically eliminated by *working in a well-ventilated room in front of an open window through which fresh air from the out doors is entering*, and *by covering the mouth of the bottle with a perforated cardboard disk during the addition of the* $Ba(OH)_2$ *at the beginning, and titration with HCl at the end of the experiment.* It is best to aërate the open bottle by holding or shaking it in the stream of air from the window just before covering it with cardboard disk and the rapid addition of the required amount of $Ba(OH)_2$ from the burette. The pasteboard disk should be held tightly over the bottle during all the manipulation and only removed just before the stopper with the small dish is inserted. A similar procedure must be carried out at the end of the experiment during titration of the remaining free $Ba(OH)_2$ with HCl.

The current of air from the window carries the exhaled air of the experimenter away from the bottles and makes the CO_2 content of the air the same in the duplicate bottles.

Sufficiently uniform manipulation in different determinations is very easily attained as the results below will show. The amount of CO_2 contained in the air in the bottle is determined by titrating with $n/100$ HCl, 15 c.c. of $n/100$ $Ba(OH)_2$ in each of two bottles. One of these samples of $Ba(OH)_2$ is titrated at once and the other at the end of an hour. The difference in the amounts of HCl is equivalent to the CO_2 of the air in each of the bottles at the beginning. To increase the accuracy three such tests are made at the same time and the average of the three taken as the true value.

The following are typical examples of tests showing the degree of uniformity.

Three samples of 15 c.c. $Ba(OH)_2$ each when titrated at once using one drop of phenolphthalein took

12.65 c.c.
12.70 c.c.
12.65 c.c.

Average 12.66 c.c. $n/100$ HCl

At the end of 1 1/2 hours three other samples of 15 c.c. of the same $Ba(OH)_2$ solution in each, took

12.35 c.c.
12.32 c.c.
12.32 c.c.

Average 12.33 c.c. $n/100$ HCl.

The amount of CO_2 absorbed was therefore equivalent to the difference or .33 c.c. $n/100$ HCl.

Several days later another set of three samples of 10 c.c. each of $Ba(OH)_2$ solution titrated at once, took

8.32 c.c.
8.32 c.c.
8.35 c.c.

Average 8.33 c.c. $n/100$ HCl.

At the end of 2 1/2 hours three corresponding samples took

8.15
8.13
8.14

Average 8.14 $n/100$ HCl.

The amount of CO_2 absorbed from the air in the bottle and during the manipulation on this day was therefore equivalent to .19 c.c. $n/100$ HCl.

The above illustrates the following facts: (*a*) The manipulation described above permits of uniform titration of the free $Ba(OH)_2$. (*b*) The amounts of CO_2 absorbed in duplicate blank bottles after allowing time for absorption, are the same. (*c*) The CO_2 absorbed from the outdoor air during manipulation and from the volume of air enclosed in the bottle may vary from day to day with different conditions but is uniform over brief periods of time. It is therefore clear that whenever necessary, proper and adequate controls to determine this amount of CO_2 can readily be provided in experiments.

If the organisms are in water in the dish, Fig. 1, *d*, then another source of variation must be taken into account, viz., possible differences in amounts of CO_2 in the water in the dish. This may be made uniform by taking equal, and as small volumes as possible, of the medium in which the organisms live, and still further reduced to small and uniform amount by using tap water as a medium as for example with *Paramecium*, Lund ('18). That the variation exists and is quite definite is indicated in the following Table I.

Table I.

The native medium was tap water in which Paramecia had been living for several hours and from which they were removed with the centrifuge. Twenty c.c. $Ba(OH)_2$ added to each bottle.

Volume of Native Medium in each Dish.			
1 C.c.	2 C.c.	3 C.c.	None.
Titrated at End of 18 Hours.			Titrated at Beginning.
C.c. *n*/100 HCl.	C.c. *n*/100 HCl.	C.c. *n*/100 HCl.	C.c. *n*/100 HCl.
20.45	20.20	20.00	21.40
20.61	20.35	19.97	21.35
20.55	20.30	20.00	21.40
Average 20.53	20.26	19.99	21.36

With air breathing organisms this source of variation is of course absent. Escape of such organisms from the dish may be prevented by a wire gauze cap placed over the small dish.

In order to determine the rapidity and completeness of CO_2 absorption by the $Ba(OH)_2$, simple and critical tests were carried out as follows. A known amount, 1 to 5 milligrams of Na_2CO_3 were weighed out in a small glass vial. This vial with the carbonate was placed in 2 c.c.–3 c.c. 50 per cent. H_2SO_4 in the dish, Fig. 1, *d*, and suspended in the bottle over the $Ba(OH)_2$. The vial was then tipped so as to allow the H_2SO_4 to decompose the carbonate, thus liberating all CO_2 from a weighed amount of Na_2CO_3. The rate and completeness of absorption of this liberated CO_2 was then tested by titrating the $Ba(OH)_2$ at different intervals of time after decomposition of the carbonate. A typical test was carried out as follows. Ten one milligram lots of Na_2CO_3 were weighed out in the glass vials as accurately as the balances and weights used would permit. One of the vials was placed in each bottle as described, over 10 c.c. $Ba(OH)_2$ solution. The time when the Na_2CO_3 was decomposed was noted and titrations made at different intervals thereafter. Controls to determine the amount of CO_2 absorbed from the air in the bottles accompanied the experiment. Results of such an experiment are given in Table II.

TABLE II.

Lots of 1 milligram Na_2CO_3 each, decomposed with 50 per cent. H_2SO_4 in the closed bottle. Ten c.c. of an approximately *n*/100 $Ba(OH)_2$ added to each bottle, Temperature 29° C.

Bottle.	Controls. No Na_2CO_3. Titrated with *n*/100 HCl at		One Milligram Na_2CO_3 Decomposed. Titrated with *n*/100 HCl at End of			
	Once.	End of 2¼ Hours.	15 Minutes.	30 Minutes.	1 Hour.	2¼ Hours.
1	8.40	8.15	6.42	6.42	6.40	6.37
2	8.38	8.17	6.35	6.50	6.34	6.45
3	8.35	8.13	6.62	6.57		
Average	8.38	8.15	6.46	6.49	6.37	6.41
c.c. *n*/100 HCl equivalent of CO_2 in air		.23				
Average c.c. *n*/100 HCl equivalent of CO_2 recovered from 1 mgm. Na_2CO_3.....			1.69	1.66	1.78	1.74
c.c. *n*/100 HCl equivalent of CO_2 expected						1.84

The absorption was evidently very rapid and nearly complete in less than thirty minutes to an hour. The average amount of CO_2 not recovered in this experiment equivalent to about 0.1

c.c. .0098*n* HCl is perhaps largely due to error in weighing such a small quantity as one milligram on the balances which were only sensitive to about 1/20 mgm. Considering the small amounts of CO_2 involved, the degree of accuracy obtainable is rather striking; the amount of CO_2 apparently not recovered in the above test being about 5 per cent. of the CO_2 liberated from 1 mgm. Na_2CO_3 or about .01 c.c. CO_2 at N.T.P. In another similar experiment using lots of 1 mgm. Na_2CO_3 about 5 per cent. *more* CO_2 was recovered than was expected from 1 mgm. of carbonate. This indicates that the error was perhaps largely one due to weighing. In one test in which was used 2 mgm. of carbonate all of the CO_2 was absorbed in less than $1^1/_2$ hours. Still another similar test to determine the rate of absorption of CO_2 from 5 mgm. of carbonate showed that at the end of fifteen minutes over 60 per cent.–70 per cent. of all the CO_2 was absorbed, and at the end of one hour all of the CO_2 was absorbed. To show that within the limits of error in weighing, the CO_2 in 2, 3 and 5 milligrams of carbonate can be practically completely recovered in relatively short periods of time by 15 c.c. weak solution of $Ba(OH)_2$ the results in Table III. are given.

TABLE III.

Bottle.	Amount of Na_2CO_3 Decomposed.		
	2 Mgms.	3 Mgms.	5 Mgms.
	Equivalent of CO_2 Recovered, in C.c. .0098*n* HCl.	Equivalent of CO_2 Recovered, in C.c. .0098*n* HCl.	Equivalent of CO_2 Recovered, in C.c. 0098*n* HCl.
1	3.84	5.45	8.88
2	3.79	5.77	8.83
3	3.95	4.86	8.80
4	3.67	5.16	8.75
5	3.75	5.29	
6	3.39	5.05	
Average	3.73	5.26	8.81
Average per milligram of Na_2CO_3....................	1.86	1.75	1.76
Expected per milligram Na_2CO_3....................	1.84		

The differences among the individual analyses were rather larger in this test than in some of the others. One and one half to two hours were allowed for absorption of the CO_2 in the above test.

The applicability of the method to measurement of CO_2, like some other methods depends upon the assumption that no other volatile acids than CO_2 are excreted by the organisms.

The carbon dioxide production by Paramecium and various small invertebrates has been measured and the results show that the method is satisfactory. For example in practical tests on Paramecium it was found that twice the number of Paramecia always produced twice the amount of CO_2, other conditions being exactly the same in two experiments. When using Paramecium the method is of course brought to a most rigid test of its usefulness, since the amounts of CO_2 produced by 2 c.c.–4 c.c. of fairly concentrated Paramecium suspension in 8 to 24 hours are much smaller than those produced by an active animal weighing one gram. Success with the method depends to a considerable extent upon a proper appreciation of the rôle of the different factors described above and care in manipulation.

Among what appear to be the special advantages of the method are the following:

1. It is simple and direct.
2. Many determinations can be made in a short time when compared with other methods.
3. The range of adaptibility is considerable and the percentage of error of the method is relatively small when the small amounts of CO_2 which can be measured, is considered.
4. The apparatus is small and many bottles can be immersed in a water bath at the same time, hence temperature can be accurately controlled in many measurements carried out at the same time.

BIBLIOGRAPHY.

Thunberg, T.
'05 Ein Microspirometer. Skand. Arch. f. Physiol., Vol. 17, p. 74.
Winterstein, H.
'13 Ein Microrespirationsapparat. Zeitschr. f. biol. Technik u. Methodik, Vol. 3, p. 246.
Krogh, A.
'16 The Respiratory Exchange of Animals and Man. Monographs on Biochemistry.
Warburg, O.
'09 Massanalytische Bestimmung kleiner Kohlensaüremengen. Zeitschr. f. physiol. Chem., Vol. 61, p. 261.

Tashiro, S.
'17 A Chemical Sign of Life. Univ. of Chicago Press.
Treadwell and Hall
'15 Analytical Chemistry, Vol. 2.
Lund, E. J.
'18 Quantitative Studies on Intracellular Respiration. Amer. Jour. of Physiol., Vol. 45, p. 351.

THE QUESTION OF THE PHYLOGENETIC ORIGIN OF TERMITE CASTES.

CAROLINE BURLING THOMPSON[1] AND THOMAS ELLIOTT SNYDER.[2]

It has recently been shown by one of the authors (C. B. T.) that the ontogenetic origin of the castes of the termite *Reticulitermes flavipes* Kol., formerly *Leucotermes flavipes*, is due to intrinsic causes, and not to the extrinsic stimuli which have long been credited with formative, indeed almost creative powers. Grassi (1893–94) and his followers described the plastic, "undifferentiated," newly hatched nymphs, upon which the action of the stimuli of food, parasites, fraternal care, etc., wrought out the highly differentiated adult castes. In favor of Grassi's hypothesis is the fact, that, in most genera, the newly hatched termite nymphs, about one millimeter long, are externally all alike. On the other hand, Grassi's hypothesis is disproved by the recent observations that termite nymphs are already differentiated at the time of hatching (Bugnion, 1912–'13, Thompson, 1917).

Bugnion states that, at the time of hatching, the soldier (nasutus) caste of the termite *Eutermes lacustris* Bugn. is sharply differentiated from the other nymphs by external structural characters. He has not worked out the differentiation of the other castes, which is, of course, a simple matter of observation, but his general conclusion, based upon this and other investigations, is that the castes of *Eutermes lacustris* originate in the embryonic period, and that the cause of differentiation is deep seated, and probably analogous with the cause of sex (Bugnion, 1912, '13).

Thompson (1917) finds that in *Reticulitermes flavipes* the newly hatched nymphs are externally all alike, but are differentiated by internal structural characters into two clearly defined types, the reproductive and the worker-soldier types, which give rise, respectively, to the three adult reproductive castes and the two adult sterile castes. The two types of newly hatched nymphs are distinguishable by four structural characters: the

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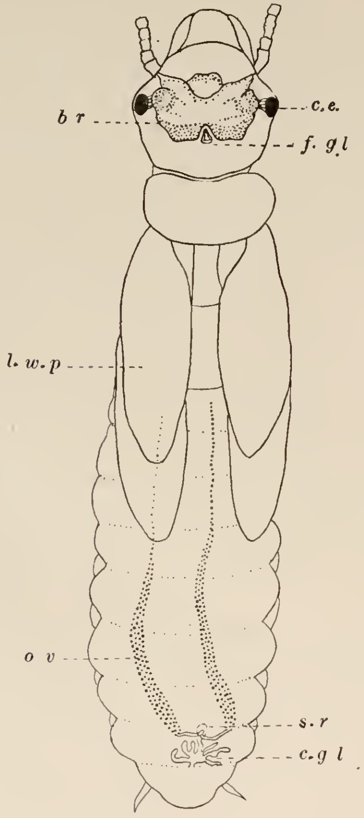

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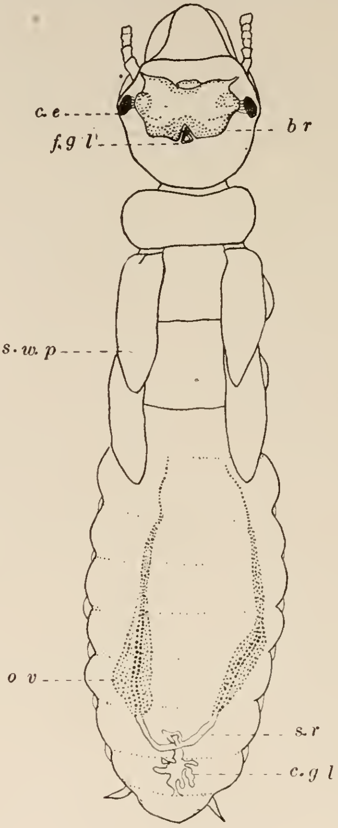

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FIG. 1. *Reticulitermes flavipes.* Female nymph of the first form, drawn from a whole mount.

FIG. 2. *Reticulitermes flavipes.* Female nymph of the second form, drawn from a whole mount. The ovaries, *ov,* which in their later development, are of secondary size, are now more swollen than in the nymph of the first form, since the ova of the second form nymphs mature at an earlier age. For the relative size of the ovaries of mature queens of the first and second forms, compare Figs. 6 and 7. It should be noted that all the parts of the reproductive system are fully formed and connected in these two fertile nymphs.

Br, brain; *c.e,* compound eye; *c.gl,* colleterial gland; *f.gl,* frontal gland; *l.w.p,* long wing pad; *s.w.p,* short wing pad; *s.r,* seminal receptacle; *ov,* ovary. Oc. 6, obj. 32 mm., drawn at stage level. Reduced one third. Drawn by C. B. T.

bulk of the brain, the relative size of brain and head, the compound eyes, and the sex organs. When the young reproductive nymphs have attained a length of 1.3–1.4 mm. other structural differences are observable that further differentiate them into two kinds of individuals which later develop into two of the three adult reproductive castes, namely: adults of the first form, with long wings, and adults of the second form, with short, scale-like wing pads. The ontogeny of the third adult reproductive caste, without wing stubs or wing pads, is yet to be worked out in *R. flavipes.* At a later period in the ontogeny—body length about 3.75 mm.—the worker-soldier nymphs differentiate into two kinds of nymphs which develop into the two sterile adult castes, the workers and the soldiers.

As a result of the discovery that the ontogenetic cause of termite castes is intrinsic, two questions arise. First, can this intrinsic ontogenetic cause be determined by cytological means, *i. e.*, by examination of the phases of ovogenesis and spermatogenesis? Second, what is the phylogenetic origin of the termite castes? It is with this second question that the present paper deals.

The phylogenetic mode of origin of the castes of termites, could it be determined, might have an important bearing upon the general question of the evolution of species. The condition of polymorphism, or the existence of several structural forms within a species, indicates that the parent or ancestral form had a tendency to vary. Two categories of variations are recognized today: (1) the variations which have arisen abruptly and are qualitatively unlike the parent condition, and which breed true to type,—"sports" of Darwin, "mutations" of DeVries, "discontinuous variations" of Bateson; (2) the "fluctuating" variations which have arisen gradually and are merely quantitative variations of the parent condition—"Darwinian variations," "fluctuations" of DeVries, "continuous variations" of Bateson. It is well known that the selectionists claim that the latter type of variation is heritable[1] while on the other hand the mutationists and the pure line school either doubt or deny that fluctuating variations are inherited.

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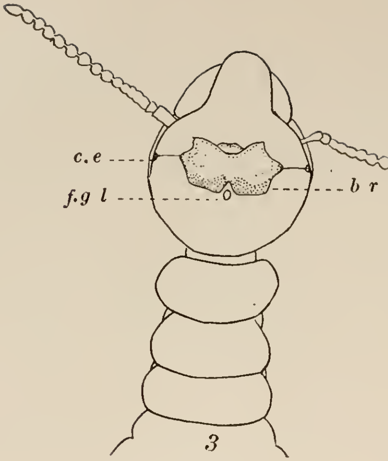
[1] Castle (1917); Jennings (1917).



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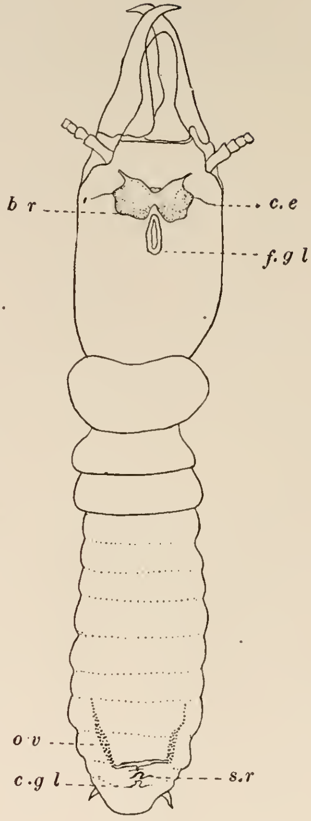

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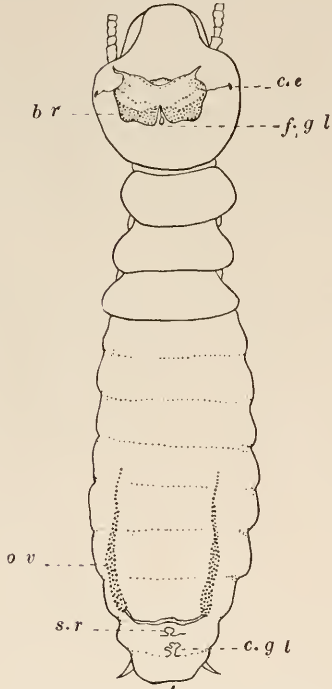

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FIG. 3. *Reticulitermes flavipes.* Mature queen of the third form, head and thorax, drawn from a whole mount.

FIG. 4. *Reticulitermes flavipes.* Female worker, adult, drawn from a whole mount.

FIG. 5. *Reticulitermes flavipes.* Female soldier, adult, drawn from a whole mount. It should be noted that the parts of the reproductive system are separate and in an embryonic condition in the sterile workers and soldiers, Knower (1901).

Br, brain; *c.e*, compound eye; *c.gl*, colleterial gland; *f.gl*, frontal gland; *s.r*, seminal receptacle; *ov*, ovaries. Oc. 6, obj. 32 mm., drawn at stage level. Reduced one third. Drawn by C. B. T.

The question that presents itself to our minds is, therefore, in which of these two categories of variations do the termite castes belong? or, in other words, are the castes of termites, both in origin and at present, to be considered as fluctuating variations or as mutations?

Several lines of approach lead toward the solution of this problem: (1) the study of fossil insects, from the Cretaceous period when termites are said to appear[1] up to the present time; (2) the comparative morphology of the order of termites; (3) exact field observations upon termite biology; (4) breeding experiments to determine the type of progeny and the results of hybridization.

None of these lines of work have been thoroughly or exhaustively investigated, and the gaps in our knowledge are very wide. There are, however, a few scattered observations, to be summarized in this paper, drawn partly from the literature of the social insects, and partly from the field and laboratory studies of the two authors, which may be of value in the attempt to solve the problem of the variations known as termite castes.

In a recent paper, Wheeler (1917) has stated his opinion that the castes of ants represent continuous, *i. e.*, fluctuating variations. This view is based upon the study of the fossil ants of the Baltic amber, and upon comparative morphological data. "In most species of ants the constant and striking structural differences between the different castes would, at first sight, suggest that such forms as the apterous females, apterous males, soldiers and workers, must have arisen as so many saltatory variations, or mutants and that they survived and secured representation in the germ-plasm, because they happened to fulfill specialized and useful functions in the life of the colony. I believe, however, that this view of the castes, at least so far as their origin is concerned, cannot be maintained, because all the available evidence points to their being merely the surviving extremes of graduated and continuous series of forms, the annectant members of which have suffered phylogenetic suppression or extinction."

Handlirsch (1908), p. 1191, states that the number of termite genera was far greater in the Tertiary period than at present.

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[1] Handlirsch (1908).

The field of termite morphology needs much revision, but a few significant facts may be stated here.

TABLE I.

THE CASTES OF *Reticulitermes flavipes* KOL.

Reproductive castes.	1. Adults of the first form	Long wings, or stubs after deälation; brain, compound eyes, and frontal gland large; mature sex organs largest of any caste; abundant pigment in skin. Aërial habit of swarming.
	2. Adults of the second form	Short wing pads; brain, compound eyes, and frontal gland smaller; mature sex organs slightly smaller; less pigment. Subterranean mode of life.
	3. Adults of the third form	Entirely wingless; brain small, compound eyes, and frontal gland vestigial; mature sex organs much smaller; still less pigment. Subterranean mode of life.
Sterile castes	4. Workers	Wingless; brain small, compound eyes and frontal gland vestigial; sex organs embryonic; no pigment in body. Subterranean mode of life. Head broader than in the first and second reproductive forms, stouter mandibular muscles.
	5. Soldiers	Wingless; brain very small, compound eyes vestigial, frontal gland large; sex organs embryonic, even smaller than in the worker; no pigment in body. Subterranean mode of life. Elongate head, long mandibles, massive mandibular muscles.

An examination of the five castes of *R. flavipes* (Figs. 1–5, 6–8, 13–15, and Table I) shows that a gradation of characters may be traced throughout the series. In the three reproductive castes (Figs. 1, 2, 3), beginning with the winged adult of the first form, or its nymph of the first form which is practically similar in structure, an increasing loss of size is to be noted in the wings, in the brain, compound eyes, and frontal gland, and in the sex organs. The darkly pigmented body and the aërial habit in swarming are found only in the adults of the first form, less pigment and a wholly subterranean mode of life in the second and third forms. In the case of the worker (Fig. 4), and to a greater extent in the soldier (Fig. 5), there is a gain as well as a loss of characters, the gains being manifested in the larger head, pigmented in the soldier, and the larger mandibles and mandibular muscles; the losses, in the body pigment, the wings, brain, compound eyes, and sex organs. The frontal gland is merely vestigial in the worker, but is highly developed in the soldier. These castes might be interpreted either as gradations in a series of continuous or fluctuating variations, or as a series of regressive mutations, *i. e.*, mutations formed by the loss of characters, comparable to the series of mutations found in *Drosophila*, Morgan et al. (1915), Morgan and Bridges (1916). Should the former prove to be the case, then transitional or intermediate forms between the existing castes should be expected, but it must be remembered that mutations also may be arranged to form a structural series, even though they may not have originated in this order.

Up to the present time rather few intermediate forms have been described. The reason for this may be either that they have been overlooked thus far, or that they do not exist. The intermediate forms known at present are as follows:

In colonies of *Termopsis angusticollis* Walker, Heath (1903) describes fertile soldiers with wing buds, which produced "normal progeny." The question might well be asked what would be "normal progeny" under these circumstances?

Soldiers with vestiges of wing pads have been noted by the two authors in several species of *Calotermes: C. occidentis* Walker, and two new species from southern Florida; and by N. Banks in

Table II.

Genus.	Reproductive Castes.			Sterile Castes.			Variations.
	With Long Wings.	With Short Wing Pads.	Wingless.	Worker.	Soldier.	Nasutus.	
Termopsis Heer	1st form.	Forms with shorter wing pads than the normal 2d form.	3d form.		Soldier.		
Calotermes Hag.	1st form.	2d form.	3d form.		Soldier.		*Calotermes* n. sp. from Fla., soldiers with abnormally shaped heads.
Neotermes Holm.	1st form.	[2]	[2]		Soldier.		
Cryptotermes Bnks	1st form.	[2]	[2]		Soldier.		
Reticulitermes Holm.	1st form.	2d form. (Also forms with both shorter and longer wing pads than the normal 2d form.)	3d form.	Worker.	Soldier.		*Reticulitermes* n.sp. from Pacific Coast, soldiers with longer heads.
Arrhinotermes Wasm	1st form.	2d form nymphs have been found, but no adults.	3d form.	Worker.	Soldier.		
Hamitermes Silv.	1st form.	2d form.	[2]	Worker.	Soldier.		
Anoplotermes F. Müll.	1st form.	2d form.	[2]	Worker.			
Eutermes F. Müll.	1st form.	2d form (in tropical species).	3d form (in tropical species).	Worker.		Nasutus.	

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[2] This caste may exist in nature but it has not yet been found.

C. minor Hagen, and other species of *Calotermes*. Vestigial wing pads are evidently of frequent occurrence in this genus although these soldiers are in general not fertile.

The Rev. F. L. Odenbach, S.J., of Cleveland, Ohio, has kindly loaned his manuscript notes from which we quote. In one of Odenbach's colonies of *Reticulitermes flavipes*, an enlarged egg-laying queen, figured in manuscript notes and referred to by Snyder (1915, p. 56), has the abdomen distended and the abdominal tergites separated, but possesses long, well developed wing pads like a nymph of the first form.

Grassi (1893) has figured a queen which, in respect to the length of the wing pads, is an intermediate between the first and second forms in the species *R. lucifugus* Rossi.

Another peculiar form has been found in a colony of *Reticulitermes* n.sp. from Montana. This specimen (a male) has the head heavily chitinized and yellow in color as in the soldier; the mandibles and labrum are like those of the worker; but the head is slightly more elongate than the typical worker head. The total length of the specimen is nearer to that of the soldier. Unfortunately the antennæ are broken, so that the question whether it is a worker or soldier can not be determined from the number of segments. After staining, the frontal gland, the compound eyes, the brain, and the sex organs were identified as those of the worker caste (see Table I.). This specimen is evidently only a worker of abnormal development, and not, as was first thought, an intermediate form between the worker and the soldier.

An argument against the view that the termite castes are mutations is the fact that the five castes are constant in their structural characters and more or less constant in their occurrence throughout the very different and widely distributed genera of the three families of termites. In the native American genera listed in Table II. the first form or reproductive adult with long wings is found in every genus. Either a second or a third form adult is probably of similar occurrence. Sometimes both are present, sometimes one or the other is said to be lacking. This may be a real absence of the caste in question, or it may be due to incomplete field data. There is no worker caste in the primitive genera *Termopsis* Heer, *Calotermes* Hag., *Neotermes* Holmg., and *Cryptotermes* Banks, but the activities of workers are carried on by the nymphs of the reproductive forms.

There is no soldier caste in the genus *Anoplotermes* Fritz Müller; this caste is also lacking in the genus *Eutermes* Fritz Müller, but here a new sterile caste has appeared: the "nasutus," a soldier-like form with a prolonged frontal process or "beak," which is often erroneously termed a soldier.

Many tropical species of termites have two types of soldiers and workers occurring within the species. The differences, however, are not marked structural ones but are quantitative, consisting mainly in the relative sizes of the two types, there being both "large" and "small" soldiers and "large" and "small" workers. These two types of worker or soldier or both, are said to occur in the genera *Rhinotermes* Hag., *Acanthotermes* Sjöst., *Termes* Linn., *Hamitermes* Silv., *Cornitermes* Wasm., *Eutermes* Fritz Müller, and *Leucotermes* Silv. In tropical species of *Eutermes* two types of nasuti, large and small, have been found. In our Nearctic termites, only two species have been seen with such differences in the soldiers. In two new species of *Calotermes* from Florida there are slight differences in the shape of the heads of soldiers *from the same colony*, and in *Reticulitermes* n. sp. from the Pacific coast there are also differences in the length of the heads of soldiers, but *from different colonies* (see Table II.).

Field Observations and Artificial Breeding Experiments.

With the collapse of the fantastic theories of Grassi and others in regard to the voluntary manufacture by the workers of "substitute" and "complemental" royal forms to replace the loss of the "true," winged, or first forms, we find ourselves at a loss to furnish the answer to the question how the different reproductive castes actually reproduce their kind today. Do the winged adults of the first form today give rise to the adults of the second and third forms as well as to the workers and soldiers? or does each fertile caste reproduce only its own fertile type together with the sterile forms?

Field observations upon species of the genus *Reticulitermes* by one author (T. E. S.) indicate that reproductive individuals of the first form probably produce the nymphs of all three reproductive castes. At the proper season of the year—before the time of swarming—nymphs of the first and second forms are commonly found together in colonies, although colonies have been found in which no nymphs of the second form have been observed. Nymphs of the third form have never yet been found, which may be due to their resemblance to the young of the other castes; there is a possibility, however, that the individuals of the third form may be fertile workers, a point to be determined by morphological data, and now being investigated by one of the authors.

Both field observations and breeding experiments seem to indicate that the second and possibly the third forms produce, in addition to the sterile workers and soldiers, only their own fertile types, and never nymphs of the first form. In other words, the second and third form reproductive adults apparently breed true to their fertile types. In some artificial colonies with parent reproductive individuals of the second form, no reproductive forms but only sterile workers and soldiers have been produced. This seems to be the case in the two following experiments.

The Rev. F. L. Odenbach received in September, 1900, a small colony of termites, *Neotermes castaneus* Burm., from Florida. He placed these insects in an artificial nest and has continued to make observations on their habits. On July 1, 1902, eggs were found in the nest. In February, 1908, about one hundred and fifty members were present in the colony. In September, 1908, and again in June, 1909, reproductive forms were observed in the nest, much larger than the other members of the colony, and some with an enlarged abdomen, the body segments appearing as prominent chitinous bands, due to distention, a characteristic of the older termite queens. From Odenbach's description these were evidently reproductive individuals of the second or third form. In December, 1910, approximately two hundred individuals were in the nest. This colony was still alive in September, 1917. Workers and soldiers, but no forms with wing pads nor any winged adults, have been produced in this colony after seventeen years of breeding.

On August 2, 1915, one of the authors (T. E. S.) received a colony of a termite (*Reticulitermes* n. sp.) from Ivywild, Colo., found in a scrub white oak, and consisting of workers, soldiers and nymphs. On November 22, 1915, three females with abdomens considerably distended and two males with slightly distended abdomens were observed in the nest. These were reproductive individuals of the second form and had grayish and yellow pigmentation in the chitinized parts. While numerous eggs have been found every month in the year in this artificial colony, maintained indoors, and while the number of workers and soldiers has increased, no forms with wing pads or wings have been produced up to December, 1918, after three years of breeding, and the colony is large—several hundred members—and healthy. The criticism can scarcely be made that there has not been sufficient time for the production of winged forms, for even in recently established incipient colonies in nature the nymphs of the winged reproductive forms are produced after eighteen months.

Heath (1903) found winged adult termites of *Termopsis angusticollis* Walker swarming from nests in which males and females of the first form were present, the nests being only two years old and containing two hundred individuals.

One of the authors (T. E. S.) has made observations on the habits of termites since 1912, mainly in the southeastern United States, but in the season of 1917 during an extensive field trip through Florida, the southwest, the Rocky Mountains and the Pacific Coast regions. It may be stated with certainty that: (1) in long-established colonies, in which large fertilized queens of the second from occurred, no nymphs or winged adults of the first form have been found; (2) in all colonies in which queens of the third form were found, no nymphs or winged adults of the first form occurred; most of these colonies, however, were all small or young, that is, they had been recently established. In one large colony of *R. flavipes* in Virginia, with seventeen third form queens present, a few nymphs of the second form were found. Very few males of the third form have yet been found in the genus *Reticulitermes*, which is possibly due to their resemblance to young nymphs of the reproductive forms or to workers, and to the fact that they are not so enlarged as the females. In the related genus *Arrhinotermes* males of the third form are of common occurrence.

The colonies of *Reticulitermes flavipes* and *virginicus* found in the southeastern United States in the spring, often contain nymphs of the second form, sometimes in large numbers, associated with either nymphs of the first form or winged sexual adults. These young reproductive types of the second form (Fig. 2) have attained their mature pigmentation at about the same time that the colonizing winged adults or reproductive types of the first form swarm, but after the swarm they are not found in the parent colonies. We may ask why they are produced and what becomes of them? They are not needed in the parent colony any more than the winged colonizing forms and it may be that they are impelled to leave the old colony by the same irresistible force that induces the swarm or flight. It may be, that, accompanied by workers and soldiers, they leave the parent colony by means of subterranean passages and establish a new colony. Since the origin of the castes is due to intrinsic causes, Thompson (1917), a certain proportionate number of these nymphs of the second form may be produced each year in long established colonies with parent first form adults. They would evidently be superfluous if the original reproductive forms of the parent colony were present, and might therefore be forced to migrate.

In certain colonies of termites, reproductive individuals of the second form occur with a small proportion of males to a large proportion of females. In the genus *Reticulitermes* as many as eight males together with thirty-two females, and fifteen males with twenty-eight females, both sexes of the second form, have been found (T. E. S.). In other colonies of *Reticulitermes* there are only reproductive individuals of the third form. Sometimes, again, a male of the first form is found with numerous (sixteen) mature females of the second form. Grassi would have described these second form females as "substitute" queens, produced by the workers to fill the place of a missing "true" queen.

It is an open question how they are to be accounted for with our present knowledge, and we have no exact data as to the progeny. In one instance three queens of the third form and one queen of the second form were found together in the same colony, but both sexes of two different mature reproductive castes have never been found in one and the same colony of *Reticulitermes*. No experiments in crossing the different forms have yet been made.

The two authors hope to undertake a thorough investigation of the termite castes, especially of the three reproductive types, with an analysis of their breeding. This will be carried on by means of field and laboratory observations and by breeding experiments which will require several years to complete.

BIBLIOGRAPHY.

Bateson, W.

'13 "Problems of Genetics." New Haven.

Bugnion, E.

'12 Observations sur les termites. Différentiation des castes. Comp. Rend. soc. biol. Paris, I., t. 72.

'12 Eutermes lacustris nov. sp. de Ceylan. Revue Suisse de Zoöl., Vol. 20.

'13 Observations sur les termites. Bull. soc. entom. France, no. 8.

Castle, W. E.

'17 The Rôle of Selection in Evolution. Journ. Wash. Acad. Sci., Vol. VII., no. 12.

Froggatt, W. W.

'96 "Australian Termiditæ," Pt. II. Proc.-Linn. Soc. New So. Wales, Pt. 4, Oct. 28.

Grassi, B., and Sandias, A.

'93 "Costituzione e sviluppo della società dei termitidi," etc. Atti dell' Acad. Gioenia di sci. nat. in Catania, Vol. VI., ser. 4, Catania.

Handlirsch, A.

'08 Die Fossilen Insekten, Leipzig.

Heath, H.

'03 The Habits of California Termites. Biol. Bull., Vol. 4.

Holmgren, N.

'06 "Studien über Südamerikanische Termiten." Zoöl. Jahrbuch, 23. Bd., Heft. 5, Jena.

'10 "Termitenstudien." 2. "Systematik der termiten." Kungl. Svenska Vetenskaps-akad. Handl., Bd. 46, no. 6. Uppsala and Stockholm.

Jennings, H. S.

'17 Observed Changes in Hereditary Characters in Relation to Evolution. Journ. Wash. Acad. Sci., Vol. VII., no. 10.

Knower, H. McE.

'01 A Comparative Study of the Development of the Generative Tract in Termites. Johns Hopkins Hospital Bull., Vol. 12, nos. 121–123.

Morgan, Sturtevant, Muller, and Bridges

'15 The Mechanism of Mendelian Heredity.

Morgan and Bridges

'16 Sex-linked Inheritance in Drosophila. Carnegie Inst. of Wash., No. 237.

Sjöstedt, Y.

'00 "Monog. der Termiten Afrikas." Kungl. Svenska Vetenskaps-Akad. Handl., Bd. 34, no. 4. Stockholm.

Snyder, T. E.

'15 Biology of the Termites of the Eastern United States, with Preventive and Remedial Measures. U. S. Dept. of Agric., Bur. Ent., Bull. no. 94, pt. II.

'16 Termites, or "White Ants," in the United States; Their Damage, and Method of Prevention. U. S. Dept. of Agric., Bur. Ent., Prof. Papr. Bull. 333.

Thompson, C. B.

'16 The Brain and the Frontal Gland of the Castes of the "White Ant," Leucotermes flavipes Kollar. Journ. Comp. Neurol., Vol. 26, no. 5.

'17 Origin of the Castes of the Common Termite, Leucotermes flavipes Kol. Journ. Morph., Vol. 30, no. 1.

Wheeler, W. M.

'17 The Phylogenetic Development of Subapterous and Apterous Castes in the Formicidæ. Proc. National Acad. Sci., Vol. 3.

DESCRIPTION OF PLATES.

FIGS. 6–9 are photographs of sections of the abdomens of termite queens. Figs. 10–12 are photomicrographs of the same sections made by John H. Paine, of the Bureau of Entomology. Figs. 13–15 are photographs of the living insects.

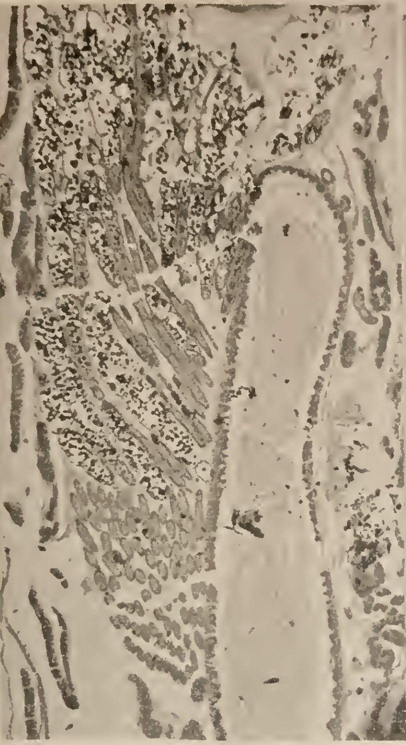
EXPLANATION OF PLATE I.

FIG. 6. *Reticulitermes flavipes.* Photograph of a section of part of the abdomen of a mature first form queen, showing that the relative extent of the ovarian tubes is greater than in either of the other reproductive castes. × 20.

FIG. 7. *Reticulitermes flavipes.* Photograph of a section of the abdomen of mature queen of the second form. A portion of the oviduct is seen with the egg tubes opening into it laterally. × 20.

FIG. 8. *Reticulitermes flavipes.* Photograph of a portion of a section of the abdomen of a mature queen. The extent of the egg tubes is less than in the other two forms, but the ova shown happen to be of the oldest stage and are therefore larger. × 20.

FIG. 9. *Eutermes morio.* Photograph of a section of the abdomen of a mature queen of the first form, showing the enormous extent of the egg tubes and the varying sizes of the ova. × 20.



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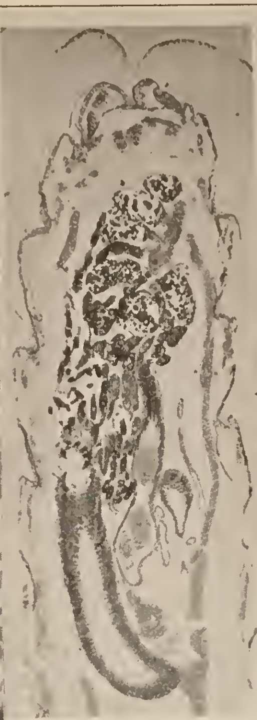

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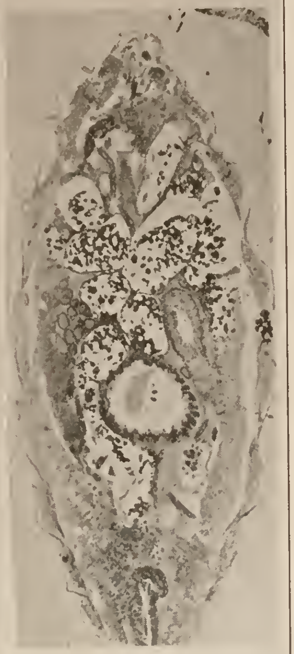

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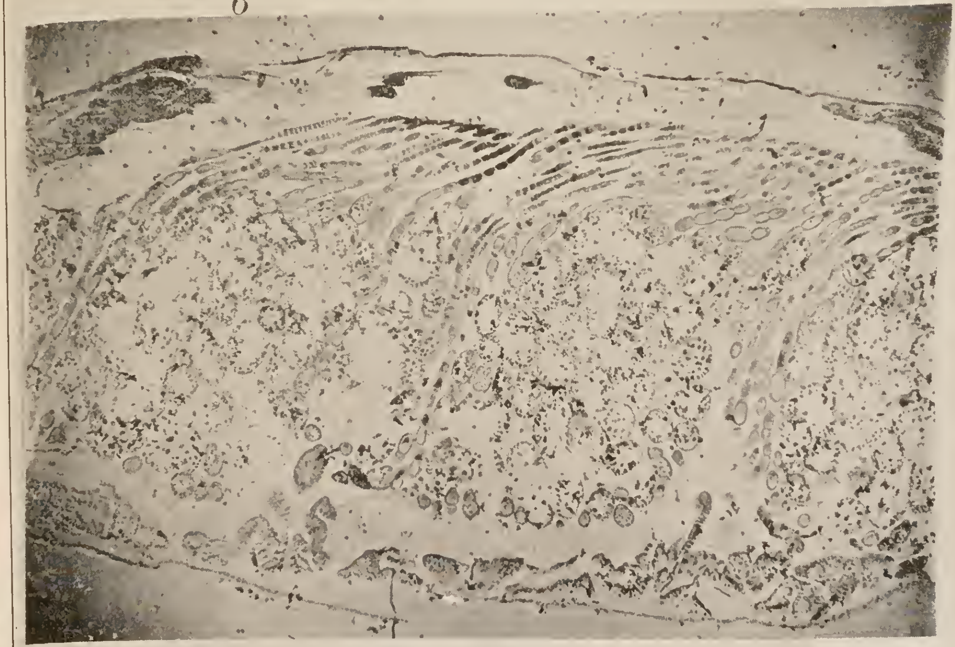

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C. B. THOMPSON AND T. E. SNYDER.

EXPLANATION OF PLATE II.

FIG. 10. *Reticulitermes flavipes.* Mature queen of the first form, ovarian tubes. Photomicrographs of a portion of the same section shown in Fig. 6. × 65.

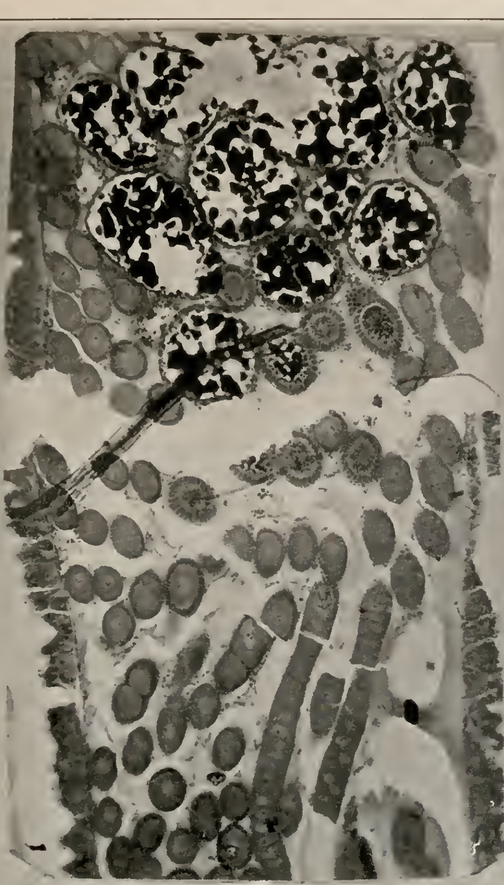
FIG. 11. *Reticulitermes flavipes.* Mature queen of the second form, ovarian tubes. Photomicrograph of a portion of the same section shown in Fig. 7. The oviduct and four entering egg tubes may be seen. × 65.

FIG. 12. *Reticulitermes flavipes.* Mature queen of the third form, ova. Photomicrograph of a portion of the same section seen in Fig. 8. × 65.

FIG. 13. *Reticulitermes flavipes.* Photograph of a mature queen of the first form. × 5.

FIG. 14. *Reticulitermes flavipes.* Photograph of a mature queen of the second form. × 5.

FIG. 15. *Reticulitermes flavipes.* Photograph of a mature queen of the third form. × 5.



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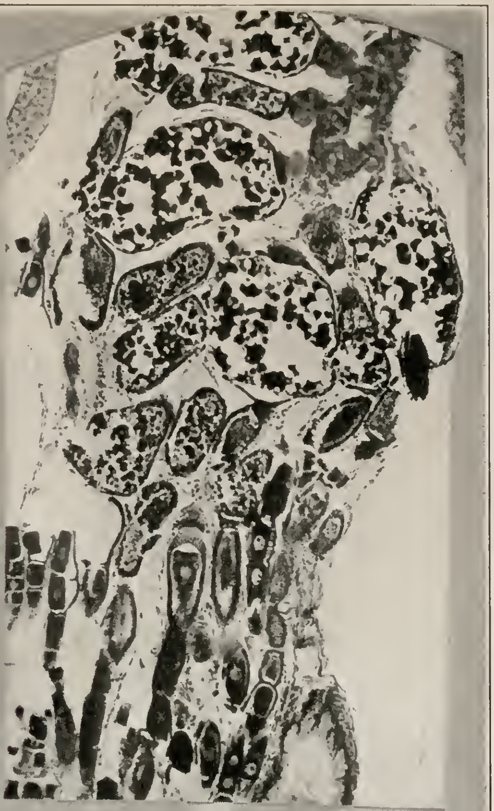

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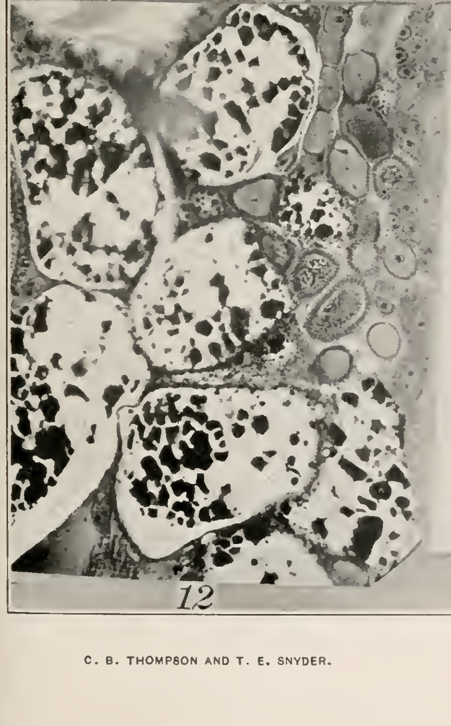

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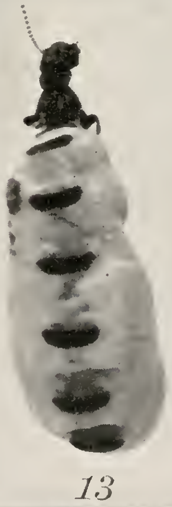

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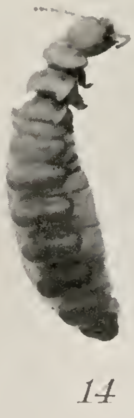

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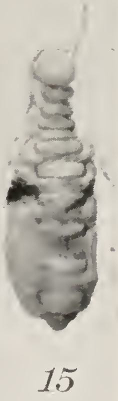

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C. B. THOMPSON AND T. E. SNYDER.

DEMONSTRATION OF THE AXIAL GRADIENTS BY MEANS OF POTASSIUM PERMANGANATE.

C. M. CHILD.

The existence of graded differences in susceptibility which are essentially quantitative rather than specific, and which show a definite relation to the physiological axes of organisms and parts has already been demonstrated for a large number of plant and animal forms by the various modifications of the susceptibility method, with a wide range of agents and conditions.[1] Additional data on other forms and with other methods, which support and further develop the original conception are as yet unpublished.

The two chief modifications of the susceptibility method, the so-called direct and acclimation methods, are concerned with the effects of different ranges of concentration or intensity of chemical and physical agents. The evidence obtained by these methods indicates that in general the regions or individuals in which the rate of oxidation or metabolism is highest are most susceptible to concentrations or intensities which either kill within a short time or are at least above the limit of acclimation or tolerance of the organism. The results of the acclimation method indicate that the regions or individuals with the highest rate of oxidation or metabolism acclimate or acquire tolerance most rapidly or most completely to concentrations or intensities which are within the limit of acclimation or tolerance. In short, the susceptibility method indicates, first, that both direct susceptibility to concentrations and intensities above the limit of tolerance and ability to acquire tolerance to lower concentrations and intensities of the same agents are associated with differences in the rate of the metabolic reactions of living protoplasm, and second, that the physiological axis *in its simplest form* is essentially a gradient in the rate of fundamental metabolic reactions. It must of course be borne in mind that the chemical reactions themselves constitute only one aspect of such a gradient: associated with them are unquestionable differences in dispersion of colloids, permeability of membranes, enzyme content or activity, electrolytic dissociation and ion content, electrical potential, etc., and the point of view determines, at least to a considerable degree, which factor or group of factors in the complex we regard as fundamental. The essential fact is that these gradients in protoplasmic condition and activity exist and that they are not only characteristic features of physiological axes, but represent the earliest and simplest condition of such axes that we have thus far been able to discover.

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[1] Child: '13, '14, '15*a*, *b*, '16*a*, *b*, *c*, *d*, '17*a*, *b*, *c*, *d*. Hyman, '16, '17.

The results given by the susceptibility method have been extended and confirmed by various other experimental methods and are not only in complete agreement with, but afford a satisfactory interpretation of a wide range of facts of observation and experiment, such, for example, as axial gradients in protoplasmic structure, in accumulation of yolk and other reserves, in rate of growth and ability to grow at the expense of other parts, in irritability and various other physiological activities.

During the past summer I have made extensive use of potassium permanganate as an agent for demonstrating the physiological gradients in protoplasm and making them directly visible as color gradients. Most of this work was done at the Puget Sound Biological Station at Friday Harbor, Wash., and I am indebted to the director, Dr. T. C. Frye and to the University of Washington for the privileges afforded me.

The Method.

In consequence of its powerful oxidizing action and the stain resulting from its reduction, potassium permanganate has long been used to some extent in histology, and certain investigators have attempted to determine with this and various other reagents the loci of reduction and of oxidation or of low and high oxygen content in cells and tissues. (See for example Golodetz and Unna, '09, P. G. Unna, '11, '13, '15, and various other papers.) The methods and results of these authors have been criticized (Oelze, '14*a*, *b*, *c*, Schmidt, '12, Schneider, '14) and their validity defended (Golodetz, '14, Unna, '15), but this controversy does not affect the well-known fact that $K_2Mn_2O_8$ is readily reduced by protoplasm to MnO_2 which produces a brown coloration of the parts affected. The use of the permanganate for the purpose of demonstrating the gradients in protoplasm is not dependent in any way on Unna's methods and results and does not assume their validity, but is based on the simple assumption that the rate of reduction of permanganate and the consequent coloration of the protoplasm may be expected to show some relation to the physiological condition of the parts concerned, as regards their metabolism, oxidative ability, reducing capacity or avidity or demand for oxygen, in short, to some of the essential factors concerned in the protoplasmic gradients.

In most previous work with permanganate relatively high concentrations have been used, *e. g.*, 1 per cent. by Unna. In such concentrations it is of course a powerful oxidizing agent and extremely toxic, killing many of the lower animals almost instantaneously. In my own work much lower concentrations have been used ranging from *m*/1,000 down to *m*/100,000, *i. e.*, 0.0316 per cent. to 0.000316 per cent.[1] Even in a concentration of *m*/1,000, however, death usually occurs, at least in some parts of the body, within a few minutes. Most of my observations have been made with concentrations of *m*/10,000 or lower, since it has been found that the regional differences in rate of staining appear more clearly in the lower concentration, where the reduction and death occur slowly and progressively, than in the higher, where they are very rapid. In fact, when the organism is killed at once or very rapidly by the permanganate the differences characteristic of the living animal do not appear at all, or are very slight, and when it is killed by some other agent before exposure to permanganate, the differences do not appear.

The results thus far obtained with permanganate have not only confirmed the results of the susceptibility methods, so far as the same forms have been used, but in many cases the great delicacy of the staining reaction has brought out very clearly differences indistinctly or uncertainly shown by the cruder susceptibility methods with lethal concentrations. In fact the permanganate method makes directly visible gradients such as the gradient in the unfertilized sea-urchin egg, which was not very satisfactorily demonstrated by the direct susceptibility methods (Child, '16*a*), but the existence of of which was inferred from the differential inhibition of development resulting from exposure of the unfertilized egg to inhibiting agents (Child, '16*c*). In various cleavage stages, hydroid planulæ, and echinoderm blastulæ and gastrulæ the color gradient is very distinct and uniform, even within the limits of a single blastomere in the earlier stages. In short the method is one of great beauty and delicacy and so entirely simple that it can readily be used for classes or lecture demonstration.

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[1] In making up solutions the formula $K_2Mn_2O_8$ with molecular wt. 316 was used. All concentrations are only approximate since solutions were not standardized.

Practically the only precaution to be observed is to provide for uniform distribution and supply of permanganate to all part of the surface of the organism. The permanganate of course gradually disappears from the solution and if the volume of solution is not very large as compared with the protoplasmic surfaces it must be renewed from time to time. Moreover, in the case of organisms lying undisturbed for any considerable time on the bottom of the dish, the surface next the glass always stains less rapidly or less deeply than other regions. These difficulties are readily avoided by slight agitation of the solution at short intervals. Ciliary movement soon ceases in all except very low concentrations of permanganate so that even with motile developmental stages and free swimming protozoa as well as with non-motile forms or stages, frequent agitation of the solution is desirable.

Besides being useful as a staining agent for demonstrating the gradients as color gradients, permanganate can also be used, in at least many cases like KNC and other agents to demonstrate differences in susceptibility as gradients in death and disintegration. Thus far it has been less used in this way than as a staining agent, but it has been found for example that in various ciliate infusoria and early developmental stages of certain forms very low concentrations such as *m*/50,000 bring about death and disintegration before staining occurs. In all such cases observed the disintegration gradient is similar to the staining gradient. In the higher concentrations there is apparently more or less coagulant or fixing action and so far as my observations go at present, the higher the concentration the less frequent is disintegration.

BRIEF STATEMENT OF RESULTS.

The use of the method thus far has fully justified the assumption on which it was based, viz., that some relation would be found to exist between the rate of reduction of permanganate by living protoplasm as indicated by the rate or intensity of the stain resulting from deposition of MnO_2 and the physiological condition of the protoplasm, particularly its oxidative activity or capacity. It has been found that the regions indicated by other experimental methods and by structural and functional characteristics of the organism as regions of high oxidation rate are most rapidly or most deeply stained in permanganate and also show the highest susceptibility as regards death and disingration of the protoplasm in the proper concentrations. Moreover, the delicacy of the method indicates clearly the existence of certain minor differences in protoplasmic and metabolic condition, which cruder methods show less clearly or not at all.

Thus far permanganate has been used as a staining agent to demonstrate the gradients in various forms as opportunity offered. These forms include algæ, protozoa, hydroid and medusa forms, eggs and early developmental stages of hydrozoa, a siphonophore, young actinians, polyclad larvae, eggs and various early developmental stages of sea urchins, and ascidian tadpoles. Some observations have also been made on elongated organs such as the tentacles and branchiæ of the polychete, *Amphitrite*. The results as regards the existence of the gradients agree in general very closely with those obtained in other ways, except as regards some minor points shown less clearly or not at all by other methods.

In the algæ examined each active physiological axis of the thallus begins to stain at the apex and the stain progresses basipetally, but in the older regions of the body irregularities appear as with other methods (Child, '16*b*, '16*d*, '17*a*). In monosiphonous branching forms such as *Callithamnium* with elongated cells, the progress of the stain along a single cell can often be observed and in such forms also the gradient in protoplasmic disintegration observed with other agents can be clearly seen.

In a study of the determination of polarity by light in the egg of the alga *Fucus vesiculosus*, permanganate and various other agents were used. As is well known, the first steps in *Fucus* development are the cell division with the membrane at right angles to the direction of incident light and the outgrowth of the rhizoid on the side of the egg away from the light. The color gradient with permanganate as well as the susceptibility gradients with KNC and various other agents showed that in the early stages the rhizoid outgrowth stains first and is most susceptible, but that after three to five days a region of rapid staining and high susceptibility begins to arise at the opposite end of the developing plant and this second region becomes and remains the region of highest rate of oxidation as indicated by these methods and forms the apical growing region of the thallus. These data are merely recorded here and will be considered more fully at another time.

Perhaps the most interesting observations among those made on plants concern a diatom of the *Navicula* group. In this form the individuals, bound together by a transparent jelly-like secretion, form an alga-like structure, a pseudothallus, often reaching a length of several centimeters, which shows a definitely directed growth, regular and orderly bifurcation, and in short possesses all the external morphological characteristics of a multiaxiate, branching alga thallus. The appearance of this pseudothallus suggests very clearly that growth and multiplication of the diatoms composing it occur chiefly in the terminal regions of the branches, in fact, it is difficult to account for the orderly axiate habitus on any other basis. In the light of these facts, it is interesting to record that this pseudothallus composed of diatoms shows in permanganate a definite basipetal staining gradient in each branch or axis, for at least several millimeters from the tip, although further basally irregularities appear. A similar gradient in susceptibility has also been observed. In this form then although protoplasmic continuity from diatom to diatom supposedly does not exist, there is a definite, orderly relation, a gradient in physiological condition along each axis, as in axiate algæ. Some sort of physiological correlation undoubtedly exists, and whatever its nature, it apparently gives rise to the same sort of order and unity as in other axiate forms. It can scarcely be supposed that in this case the correlation is accomplished by chemical substances given off by the individual diatoms, and the only other possibility is a transmissive relation of some sort, probably electrical in character (see R. S. Lillie, '17).

Among the protozoa observations have been made on *Noctiluca*, *Paramecium*, *Stentor* and *Spirostomum*. In *Noctiluca* the first traces of color appear on the surface of the membrane in the mouth region and the color spreads gradually over the membrane. The entoplasm later contracts and disintegrates into droplets before staining, but some slight indications of a basipetal gradient appear in this disintegration. The thickened flagellum of *Noctiluca* shows a well marked basipetal gradient.

In *Stentor* the color gradient in the ectoplasm is essentially the same as the susceptibility gradient already described (Child, '14), *i. e.*, basipetal, but the permanganate indicates a slight degree of "dorso-ventral" difference in that the staining progresses basipetally more rapidly on the oral than on the opposite side of the body. This difference along different meridians of the body has not been observed in susceptibility agents.

Spirostomum shows an ectoplasmic basipetal gradient both in color and in disintegration in permanganate, a short gradient in the opposite direction appearing at the basal end in a varying percentage of individuals. Other agents give essentially similar results. The secondary gradient in this form is very probably associated with the reversibility in the direction of locomotion which this species shows to a very high degree and with stimulation or irritation by the reagent for it apparently occurs only in those individuals which contract strongly and repeatedly in the solution.

In *Paramecium* the first effect of permanganate is, even in very low concentrations, a contraction of the ectoplasm beginning, and most extensive at the anterior end. This results in approach to spherical form, and in the lower concentrations the body often bursts either in the "anal" region or near one of the vacuoles.

In the higher concentrations a slight basipetal color gradient appears, but here again the posterior end commonly also stains almost as rapidly as the anterior, suggesting the existence of a secondary region of high activity there. Earlier work with susceptibility agents indicated a slight basipetal gradient in the majority of individuals, but the results were not as uniform as in many other species (Child, '14). It seems at least possible that certain irregularities in disintegration observed in the earlier work depend in part on the existence of a second region of rather high susceptibility in the posterior part of the body.

Five genera of hydromedusæ, *Phialidium*, *Æquorea*, *Mitrocoma*, *Sarsia* and one undetermined genus, tested repeatedly in permanganate, have shown in all cases a more rapid staining of the ectoderm of the subumbrella, than of the exumbrella. The difference is apparently greater in young than in old animals. These results agree with those of McClendon ('18) on oxygen consumption in the scyphomedusa *Cassiopea xamachana*. He found that the oxygen consumption of the subumbrella, exclusive of the manubrium is more than four times that of the exumbrella in the resting animal, with a still greater difference when pulsations occur. Each medusa tentacle also shows a basipetal color gradient.

In the colonial hydroids, *Bougainvillea*, *Obelia*, *Gonothyria* and one undetermined campanularian genus each hydranth body and each tentacle shows a basipetal color gradient, and with low concentrations a colony gradient also appears, *i. e.*, in a long stem with primary, secondary and perhaps tertiary branches the hydranths and growing tips of the apical region of the whole colony stain more rapidly than those of the basal region and the same differences appear on each of the longer primary branches. This colony gradient is clearly visible to the naked eye when the whole stem with its hydranths is placed in water after staining.

A basipetal color gradient also appears in the stems of the colony, the growing tips always staining much more rapidly and deeply than more basal regions. As regards these stems, however there is of course the possibility that the permanganate may penetrate the thinner perisarc of apical growing regions more readily than the thick perisarc of more basal levels, though, as a matter of fact, penetration of the perisarc apparently occurs almost at once, and the differences in rate and depth of staining at different levels appear to be very much greater than the differences in perisarcal penetration.

The developmental stages of the egg of the hydromedusa, *Phialidium* from the ovarian egg to the hydroid have been examined with permanganate. In the ovarian egg, isolated by teasing, the region of the egg next to the free surface of the gonad stains most rapidly, with a color gradient from this region to the basal egg-pole. This gradient persists during fertilization, cleavage and to the advanced planula stage, the region of most rapid staining being the apical end of the embryo. In the late, elongated planula, just before attachment, a second region of rapid staining arises at what was originally the basal end, the planula attaches itself by its apical end, and the first hydroid arises as a bud from this second region of rapid staining at the basal end. The fact of attachment of the planula by the apical end and the development of the first hydranth from the original basal end has long been known to embryologists, and the MnO_2 color gradients merely serve to give some indication of the physiological conditions concerned. Apparently the first hydranth is physiologically a bud, a process of agamic reproduction in the planula, resulting from physiological isolation at the basal end, a process similar to the development of a second hydranth at the tip of the stolon in *Tubularia* (Child, '15*b*, pp. 91–92) the development of segments in annelids (Hyman, '16 Child, '17*d*), and many other reproductive processes in other forms (Child, '15*b*, Chapter V.). These results on hydrozoa are in agreement with those obtained by other methods, except that as regards unfertilized eggs, cleavage stages and general colony, gradients, the permanganate results are more distinct and definite.

The color gradients have been determined in one species of siphonophore belonging to the family Monophyidæ, a form with a single, elongated nectocalyx, from one side of which the stem or cœnosome bearing the groups of zoöids arises. The nectocalyx, which is of course a modified medusa, is morphologically bilaterally symmetrical and shows in permanganate, not only a more rapid staining of the subumbrella than of the exumbrella like other hydromedusæ, but also a beautiful and striking bilaterality in the staining gradient, the side opposite the origin of the colony stem staining most rapidly, the stem side least rapidly. This difference is also visible with susceptibility methods, but less distinctly. In the groups of zoöids on the stem each zoöid, both nutritive and medusoid, shows a basipetal color gradient and the medusoid zoöids also show a bilateral gradient similar to that of the nectocalyx. In fact, these medusoids along the stem may later become nectocalyces, for each group, consisting of one nutritive, one medusoid zoöid, a group of tentacles and a bract, may become free and develop into a new siphonephore colony. The bilaterality of the color gradient in this form is a very striking feature.

Young actinians including the genus *Peachia* which is parasitic on hydromedusæ during its earlier life-history, and several other undetermined forms showed a very distinct basipetal color gradient in tentacles and body, and with certain concentrations a similar disintegration gradient.

In various embryonic and larval stages of polyclads, a beautiful basipetal color gradient appeared uniformly.

Unfertilized eggs of the sea urchin, *Strongylocentrotus franciscanus* show uniformly a very definite color gradient, although in the absence of definite landmarks it is not absolutely certain that this gradient is basipetal. However, the egg after fertilization and during cleavage does show a distinct basipetal gradient, even in each of the first two blastomeres, and these can be little doubt that this gradient is identical with that of the unfertilized egg. This gradient is also present in blastula and gastrula and is identical with the susceptibility gradient (Child, '16*a*).

The elongated tentacles and the branched branchial apparatus of the annelid, *Amphitrite* both exhibit very marked basipetal color gradients and in tadpoles of the solitary ascidian, *Corella willmeriana* the growing tail stains most rapidly at the tip with a definite gradient to the base, and the three papillæ of attachment which in this form grow out into temporary stolons during metamorphosis, but are later resorbed, are also regions of rapid staining. On the body of the larva staining progresses from the papillae more rapidly on the dorsal than on the ventral side.

These miscellaneous data on widely separated groups include the chief results obtained thus far with permanganate. It should perhaps be stated that in every case, except certain hydroids, the siphonophore and actinians at least ten individuals, and in the case of eggs and embryos hundreds or thousands were used, and the experiments were repeated at different times. With some of the hydroids only five or six branching stems from different colonies were used with the same results in all cases. In the case of the siphonophore three colonies were used, and since the results fully confirmed those obtained by other methods, and in each colony the same result could be observed in the different groups of zoöids, larger numbers were considered unnecessary. Of the actinians three to six individuals of each form tested were used, the uniformity of result in all the species making large numbers unnecessary.

Discussion.

The data briefly recorded above are sufficient to show that axial gradients in rapidity of staining with MnO_2 produced by the reduction of permanganate in low concentrations are at least of very wide occurrence in axiate organisms and organs. Moreover, in every case where data have already been obtained by other methods these have been confirmed by the color gradient, and in some cases slight symmetry gradients which were not visible or were indistinct with other methods have been clearly seen.

For various forms, including protozoa, medusa planulæ, echinoderm eggs and embryos and ascidian tadpoles, it has been demonstrated that the color gradient appears only when the living animals are brought into permanganate. When they are first killed without disintegration by some other agent, *e. g.*, mercurie chloride, alcohol, etc., and then brought into permanganate, staining is uniform, except where structural differences are concerned, and the gradient does not appear. Like the susceptibility gradients, the color gradients are thus features of living axiate organisms. This is undoubtedly true for all forms, but its truth has not been proved for all.

Only one further point need be considered here, viz., the question whether the color gradients are merely a result of differences in rate of penetration of permanganate, in consequence of differences in permeability of different regions of cell or body, or whether they are more directly related to metabolic conditions in the protoplasm. With respect to this question it may be noted first that the color gradient begins to appear as a superficial gradient, apparently resulting from the deposition of MnO_2 on the cell surface. This is particularly evident in single cells, *e. g.*, *Noctiluca*, *Stentor*, eggs and blastomeres, where in optical section it can be seen that no appreciable penetration has occurred when the gradient has already begun to appear. Of course it cannot be denied that in such cases the permanganate has already penetrated a membrane of molecular thickness, but it certainly has not penetrated the visible structural membrane of the cell. In the protozoa the gradient is wholly or almost wholly limited to the ectoplasmic layer, but in eggs it apparently exists to some degree throughout the cytoplasm but appears first superficially. The facts seem at least to indicate that differences in a purely physical permeability of the cell membrane will not account for the color gradient any more than for the susceptibility gradient or the gradients in differential inhibition and acceleration of development.

As regards the question of permeability, however, it is doubtful whether we can isolate it as a purely physical condition from the chemical activity going on in the living protoplasm. Recent investigation indicates more and more clearly that such isolation is impossible. Without going into the matter at length, it is of interest to note that Osterhout has conceived changes in permeability in terms of chemical reaction, that R. S. Lillie in his later papers has emphasized the importance of metabolic reactions in relation to permeability and that recently Crozier ('18*a*, *b*) has concluded that for acids and alkalies the essential factor in stimulation is not an increase in physical permeability by depolarization, but rather a chemical reaction between the agent and some constituent of the receptor cell. And finally, even if we accept a purely physical theory of permeability and assume that the axial gradients in susceptibility to various agents and rate of staining by permanganate represent primarily gradients in permeability, there can be no doubt that such relatively permanent, localized and graded differences in permeability in such definite relation to the physiological axes, must be more or less closely associated with differences in metabolic condition, and that in general and within certain physiological limits we may expect to find a higher rate of oxidation associated with a region of higher permeability. Even on this basis then, the axial gradients in susceptibility, color, etc., would serve as indicators of metabolic gradients. But that a purely physical permeability of chemically inert membranes is not the primary factor in this relation is becoming increasingly evident, not merely in the recent modifications in the conception of permeability, but in the facts of susceptibility themselves. For example, differences in permeability of different cells or cell regions along an axis to neutral red and certain other "vital" dyes are usually slight and often inappreciable, although in some cases a distinct permeability gradient can be seen. Nevertheless axial gradients in susceptibility to these agents are very distinct, even within the limits of single cells, whether a gradient in penetration is distinguishable or not. Certainly physical permeability of the cell membrane is not the primary factor in such susceptibility gradients. Again, in the processes of acclimation and recovery that region of an axis which is most susceptible to the agent in high concentration or intensity undergoes acclimation most rapidly or completely in low concentrations or intensities, or recovers most rapidly and completely after temporary exposure within certain limits. Obviously the axial gradients of acclimation and recovery are very directly dependent upon metabolic rate in protoplasm and purely physical differences in permeability are of minor importance.

In the light of all the facts, then, I believe we are justified in concluding as regards the color gradients resulting from the protoplasmic reduction of permanganate; first, that they are indications of fundamental quantitative differences in the physiological condition of protoplasm, second, that such condition is very intimately related to the rate of oxidation, third, that the quantitative graded differences thus indicated represent the physiological axis in its simplest form.

SUMMARY.

The axial gradients in many plants and animals can be very clearly and beautifully demonstrated as color gradients by the use of low concentrations of $K_2Mn_2O_8$, the color being due to the reduction of the permanganate to MnO_2 by protoplasm.

Axial gradients have been demonstrated by this method in all axiate organisms thus far examined including several algæ, a diatom pseudothallus, four species of protozoa, various hydroids, hydromedusae and their developmental stages, a siphonophore, actinians, developmental stages of polyclads and echinoderms, axiate appendages of polychetes, and ascidian tadpoles.

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REFERENCES.

Child, C. M.

'13 Studies on the Dynamics of Morphogenesis, etc. VI., The Nature of the Axial Gradients in Planaria and their Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwicklungsmech., XXXVII.

'14 The Axial Gradient in Ciliate Infusoria. BIOL BULL., XXVI.

'15a Axial Gradients in the Early Development of the Starfish. Am. Jour. Physiol., XXXVII.

'15b Individuality in Organisms. Chicago.

'16a Axial Susceptibility Gradients in the Early Development of the Sea Urchin. BIOL BULL., XXX.

'16b Axial Susceptibility Gradients in Algæ. Bot. Gaz., LXII.

'16c Experimental Control and Modification of Larval Development in the Sea Urchin in Relation to the Axial Gradients. Jour. of Morph., XXV.

'16d Further Observations on Axial Susceptibility Gradients in Algæ. BIOL. BULL., XXXI.

'17a Susceptibility Gradients in the Hairs of Certain Marine Algæ. BIOL. BULL., XXXII.

'17b The Gradient in Susceptibility to Cyanides in the Meridional Conducting Path of the Ctenophore *Mnemiopsis*. Am. Jour. Physiol., XLIII.

'17c Experimental Alteration of the Axial Gradient in the Alga, *Griffithsia bornetiana*. BIOL. BULL., XXXII.

'17d Differential Susceptibility and Differential Inhibition in the Development of Polychete Annelids. Jour. of Morph., XXX.

Crozier, W. J.

'18a On Sensory Activation by Alkalies. Am. Jour. Physiol., XLV.

'18b Sensory Activation by Acids. Am. Jour. Physiol., LXV.

Golodetz, L.

'14 Die Darstellung der Reduktionsorte und Sauerstofforte der Gewebe. Zeitschr. f. wiss. Mikr., XXX.

Golodetz, L., and P. G. Unna.

'09 Zur Chemie der Haut. III., Das Reduktionsvermögen der histologischen Elemente der Haut. Monatshefte f. prakt. Dermatol., XLVIII.

Hyman, L. H.

'16 An Analysis of the Process of Regeneration in Certain Microdrilous Oligochetes. Jour. Exp. Zoöl., XX.

'17 Metabolic Gradients in Amœba and their Relation to the Mechanism of Amœboid Movement. Jour. Exp. Zoöl., XXIV.

Lillie, R. G.

'17 The Formation of Structures Resembling Organic Growths by Means of Electrolytic Local Action in Metals and the General Physiological Significance and Control of this Type of Action. BIOL. BULL., XXXIII.

McClendon, J. F.

'18 Direct and Indirect Calorimetry of *Cassiopea xamachana*. Jour. Biol. Chem., XXXII.

Oelze, F. W.

'14a Uber die färberische Darstellung der Reduktionsorte und Oxydationsorte in Geweben und Zellen. Arch. f. mikr. Anat., LXXXIV.

'14b Die Histologie der Oxydations- und Reduktionsorte. Zeitschr. f. wiss. Mikr., XXXI.

'14c Die Darstellung der Reduktionsorte und Sauerstofforte der Gewebe. Zeitschr. f. wiss. Mikr., XXXI.

Schmidt, M.

'12 Die Reduktions- und Sauerstofforte der pflanzlichen Gewebe. Verh. d. naturwiss. Vereins Hamburg, 3 Folge, XIX.

Schneider, H.

'14 Über die Unnaschen Methoden zur Feststellung von Sauerstoff und Reduktionsorten und ihre Anwendung auf pflänzliche Objekte. Zeitschr. f. wiss. Mikr., XXXI.

Unna, P. G.

'11 Die Reduktionsorte und Sauerstoffsorte des tierischen Gewebes. Arch. f. mikr. Anat., LXXVIII.

'13 Biochemie der Haut. Jena.

'15 Die Sauerstoffsorte und Reduktionsorte. Arch. f. mikr. Anat., LXXXVII.

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BIOLOGICAL BULLETIN

ON THE REVERSIBILITY OF THE HELIOTROPISM OF ARENICOLA LARVÆ BY CHEMICALS.[1]

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I. Introductory.

Loeb discovered in 1893 that the normal heliotropism of *Polygordius* larvæ and marine copepods could be reversed by changes of the temperature and of the concentration of sea-water.[2] He also found in 1904 that fresh-water crustaceans which were naturally negative or indifferent to light could be made positively heliotropic by chemicals.[1] Since then, Holmes.[2] Minkiewicz,[3] Drzewina,[4] Jackson,[5] Moore,[6] Ewald,[7] and others have shown many examples of the reversibility of heliotropism in animals by chemicals, temperature, etc. R. S. Lillie,[8] Mast,[9] and the writer[10] have also observed the reversing effect of chemicals on the positive heliotropism of *Arenicola* larvæ. These observations, however, were rather incidental and not extensive,

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[1] From the Marine Biological Laboratory, Woods Hole, Mass., and the Physiological Laboratory, University of Minnesota, Minneapolis.

[2] Loeb, Jacques, *Pflüger's Arch.*, Bd. 53, p. 81, 1893.

In this paper, the writer shows quantitatively that the normal heliotropism of *Arenicola* larvæ can be reversed by various chemicals and by higher or lower temperatures than normal. And it will be seen that those chemicals which produce artificial parthenogenesis in sea-urchin and other eggs have also a reversing effect on the heliotropism of *Arenicola* larvæ. This parallelism between the reversal of the normal heliotropism of the larvæ and artificial parthenogenseis is striking as regards all chemicals used by the writer except inorganic acids.

The experimental work was done partly under the direction of Professor R. S. Lillie in the physiological department of the Marine Biological Laboratory at Woods Hole, Mass., U. S. A., during the summer of 1914. Publication has been delayed chiefly on account of the writer's return to Japan. The writer wishes here to acknowledge his indebtedness to Prof. Ralph S. Lillie and Prof. Elias P. Lyon for their valuable suggestions and criticism of the work and manuscript. His thanks are due also to Director Frank R. Lillie, who gave him the use of a research room in the laboratory.

II. Material and Methods.

The larvæ of the marine annelid, *Arenicola cristata*, were used for this work. The procedure of obtaining the larvæ was exactly the same as described in the writer's previous paper.[1] The free-swimming larvæ, just after they leave the egg-strings at the swarming stage, are strikingly positive to light. They are, therefore, very favorable for work of this kind.

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[1] Loeb, Jacques, Univ. of Calif. Pub., Physiol., Vol. 2, p. 1, 1904.

[2] Holmes, Samuel J., *Jour. Comp. Neur. and Psychol.*, Vol. 15, 305, 1905.

[3] Minkiewicz, R., *Arch. d. Zoöl. Exp. et Gén.*, t. 7, p. 37, 1907.

[4] Drzewina, Anna, *C. R. Soc. Biol.*, Vol. 71, 555.

[5] Jackson, H. H. T., *Jour. Comp. Neur. and Psychol.*, Vol. 20, p. 259, 1910.

[6] Moore, A. R., *Science*, N. S., Vol. 38, p. 131.

[7] Ewald, Wolfgang F., *Jour. Exp. Zoöl.*, Vol. 13, p. 591, 1912.

[8] Lillie, Ralph S., *Am. Jour. Physiol.*, Vol. 24, p. 14, 1909.

[9] Mast, Samuel O., "Light and the Behavior of Organisms, xi + 410 p., 1911.

[10] Kanda, Sakyo, *Am. Jour. Physiol.*, Vol. 35, p. 162, 1914.

The methods of experimentation were simple. At each trial, four 100 c.c. beakers, one for control with normal sea-water at room temperature, a second with a treated sea-water at room temperature, a third with the same (treated) sea-water at higher temperature, and a fourth with the same (treated) sea-water at lower temperature, were used. Fifty c.c. of liquid were used in each beaker. Each beaker was set in a larger vessel (finger bowl), containing sea-water of the same temperature as the beaker. When all the beakers were ready and fairly constant at the desired temperatures, the larvæ taken with a small pipette from a very dense aggregation were distributed as uniformly as possible in each beaker.

Diffuse light came horizontally from one south window only. Care was taken against reflected light by using folded black cloth under the beakers and on a long board that was placed at the north side of the dishes.

III. Experimental.

Recent discoveries in the reversing effect of temperature of normal sea-water on the normal heliotropism of various animals had made it desirable to examine this phenomenon in *Arenicola* larvæ and to see whether there is any temperature coefficient when chemicals were added to it. In some experiments this was fairly illustrated, as far as time-relation was concerned, but others were not so successful as expected, possibly on account of the entrance of other factors.

1. *Effects of Temperature.*

Mast states that he did not succeed in producing a reversal of the positive heliotropism of *Arenicola* larvæ by changing the temperature of sea-water.[2] This was not the case in the writer's experiments. Mast perhaps used old larvæ instead of very young ones. The sensitivity of the larvæ to changes of medium varied greatly according to their age. It was impossible to get larvæ uniformly young. This was the reason why one could not get a reversal of all larvæ treated even with the best agents. There was always one or two per cent. of exceptions. Table I. gives the summary of several series of the writer's experiments.

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[1] Kanda, *loc. cit.*

[2] Mast, *loc. cit.*

TABLE I.

EFFECTS OF TEMPERATURE.

50 C.c. Sea-water.	Immed. After the Treatment.	1 Min. After the Treatment.	5 Min. After the Treatment.	10 Min. After the Treatment.	25 Min. After the Treatment.
31° C.	Practically all positive	Some became negative.	About 50 per cent. became negative.	Same as before.	Same as before.
11° C.	Almost motionless.	Many became positive.	Very few became negative.	About 15 per cent. became negative.	About 20 per cent. became negative.
21° C. (Control)	Practically all positive.	Practically all on the window side	Same as before.	Same as before.	A few became negative.

It was peculiar that high and low temperatures had the same reversing effect on the larvæ, although cold was slower and less effective. Loeb[1] showed in *Polygordius* larvæ a phenomenon similar to this.

2. *Effects of Hypertonic and Hypotonic Sea-water.*

(*a*) *Effect of Hypertonic Sea-water.*—R. S. Lillie[2] and Mast[3] observed that hypertonic and hypotonic sea-water produced the reversal of the normal heliotropism in *Arenicola* larvæ. The writer tested the effect of hypertonic sea-water by mixing 15 c.c of one mol NaCl solution and 35 c.c. of natural sea-water at room temperature, 22° C. He found that about 20 per cent. of larvæ became negative. He did not, however, succeed in producing a reversal in this medium by raising or lowering temperature, *i. e.*, 32° C. or 12° C. The larvæ in this mixture were very sluggish at these temperatures.

In the mixture of 0.85 c.c. of m-KCl solution and 50 c.c. of natural sea-water at room temperature, 22° C., it was found that 30 minutes after the treatment about 35 per cent. of the larvæ became negative to light. Besides sodium and potassium chlorides, m-$CaCl_2$, m-$MgCl_2$, and 2m-$MgSO_4$ were tested without success. Temperature experiments also failed in all these mixtures of hypertonic sea-water.

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[1] Loeb, J., *loc. cit.*

[2] Lillie, R. S., *loc. cit.*

[3] Mast, S. O., *loc. cit.*

(*b*) *Effect of Hypotonic Sea-water.*—The mixture of 30 c.c. of natural sea-water and 20 c.c. of distilled water (which was the optimum dilution) was very remarkable in producing a reversal of heliotropism. The effect was temporary only. The results of several series are summarized in Table II. It is also interesting to note the difference in the time required to produce the maximum results at the three temperatures 12°, 22°, 32°. A Q_{10} value of about 2 is indicated for the reversive process.

TABLE II.

EFFECT OF HYPOTONIC SEA-WATER.

Solution.	Temperature.	Best Result Obtained.	Result.
30 c.c. sea-water + 20 c.c. dist. water.	32° C.	10 min. after the treatment.	About 90 per cent. became negative.
30 c.c. sea-water + 20 c.c. dist. water.	22° C. (room temp.)	20 min. after the treatment.	About 90 per cent. became negative.
30 c.c. sea-water + 20 c.c. dist. water.	12° C.	40 min. after the treatment.	About 90 per cent. became negative.
50 c.c. nat. sea-water (control).	22° C. (room temp.)	1/2 min. after the treatment.	Practically all positive.

The larvæ lived over 15 days and grew well in this hypotonic sea-water.

3. *Effects of Isotonic Salt Solutions.*

A question arose whether these reversing effects of hypertonic and hypotonic sea-water were simply osmotic or not. This idea was tested by using isotonic salt solutions. In all of these series the problem of temperature was omitted from consideration. The results are given in Table III.

Since the sodium, lithium, potassium or ammonium chloride and sulphate solutions that were used were all isotonic with sea-water, it is evident that the reversing effect of these salts is not simply an osmotic one. And since the action of these eight salts was different from that of calcium or magnesium chloride

TABLE III.

EFFECT OF ISOTONIC SALT SOLUTIONS.

Temp. of Mixt.	Mixture of Isot. Sol. and Sea-water.	Time After the Treatment for Maximum Effect.	Per Cent. of Negative Heliotropism Produced.
20° C.	1.5 c.c. 0.52 m. NH_4Cl + 50 c.c. sea-water.	About 1 hr.	About 90 per cent.
20° C.	2 c.c. 0.52 m. KCl + 50 c.c. sea-water.	About 20 min.	About 70 per cent.
21° C.	3 c.c. 0.54 m. $LiCl$ + 50 c.c. sea-water.	About 25 min.	About 50 per cent.
21° C.	30 c.c. 0.52 m. $NaCl$ + 20 c.c. sea-water.	About 1 hr.	About 30 per cent.
21° C.	20 c.c. 0.35 m. $MgCl_2$ + 30 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
21° C.	20 c.c. 0.35 m. $CaCl_2$ + 30 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
20° C.	1 c.c. 0.46 m. $(NH_4)_2SO_4$ + 50 c.c. sea-water.	About 2 hrs.	About 90 per cent.
20° C.	1 c.c. 0.46 m. K_2SO_4 + 50 c.c. sea-water.	About 40 min.	About 75 per cent.
21° C.	1.5 c.c. 0.47 m. Li_2SO_4 + 50 c.c. sea-water.	About 1 hr.	About 70 per cent.
21° C.	20 c.c. 0.46 m. Na_2SO_4 + 50 c.c. sea-water.	About 2 hrs.	About 60 per cent.
21° C.	12 c.c. 0.9 m. $MgSO_4$ + 50 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
21° C.	50 c.c. nat. sea-water.	About 1/2 m.–5 hrs.	Pract. all positive.

and sulphate solutions, the reversing effect of the former eight salts would seem to be specific. The chlorine and sulphate ions in these twelve salts being the same, the specific action of isotonic sodium, lithium, potassium or ammonium chloride and sulphate solutions seems to be due essentially to cations instead of anions. The effective order of these cations may be expressed as follows:

$$Na^+ < Li^+ < K^+ < NH_4^+$$

That a yellow pigment contained in the cells of the larvæ diffused out into the medium, when treated with the mixture of isotonic Na_2SO_4 or NH_4Cl solution and sea-water, was striking. This took place between eight and ten minutes after the treatment A similar exit of pigment was first observed by Lillie in isotonic $NaCl$, KCl and NH_4Cl solutions, and has been generally regarded as indicating an increased permeability of the cell membrane.

A question arises whether the reversal of positive heliotropism of the larvæ was due directly to the diffusion of cations into the cells as indicated above or not. In the cases of Na_2SO_4 and

NH_4Cl solutions, the most notable exit of pigment from the larvæ—the sign of increased permeability according to Lillie—took place at about ten or twenty minutes after the treatment. In the case of Na_2SO_4 solution about 5 per cent. of the larvæ became negative at about ten minutes, while in NH_4Cl solution the larvæ were evenly distributed at about 20 minutes after the treatment. The maximum reversal in the former was produced at about two hours and in the latter at about one hour after the treatment. The reversing effect of these two salts, therefore, seems to be too slow to be regarded as the direct action of ions which have diffused into the larvæ. At the same time, the effect is not on the boundary membrane alone, because these salts must have modified and penetrated the membrane long before the change of reaction in the larvæ occurred. The larvæ treated with Na_2SO_4 and NH_4Cl stick tightly to the window side of a beaker 7 or 8 minutes after the treatment. This fact may explain the difficulty. That is to say, if they had been free in swimming, they might have reversed their heliotropic reaction more promptly than they actually did.

As to the way in which the behavior of organisms is modified by electrolytes such as salts, we are as yet in the dark. At any rate, the reversal of positive heliotropism of the larvæ by these electrolytes is an insufficient index by which to gain an understanding of the mechanism of their physical or chemical influence on the behavior of organisms.

4. *Effects of Artificial Sea-water.*

The artificial sea-water that the writer used was a van't Hoff mixture of the following composition:

0.52 m. Nacl	100 c.c.
0.52 m. KCl	2.2 c.c.
0.35 m. $CaCl_2$	1.5 c.c.
0.35 m. $MgCl_2$	7.8 c.c.
0.90 m. $MgSO_4$	3.8 c.c.

To this a trace of $NaHCO_3$ was added as Loeb advised. This artificial sea-water with or without the alkali affected little the positive heliotropism of the larvæ. About five per cent. of them showed negative heliotropism. Besides these, a few larvæ in this solution swam about at the bottom of the beaker. About 90 per cent. or more of the larvæ were always positive to light.

5. *Effects of Hypertonic Sodium and Potassium Chloride Solutions Added to the Artificial Sea-water.*

These effects were remarkable. In the mixture of 15 c.c. m-NaCl and 35 c.c. of the artificial sea-water, about 90 per cent. of the larvæ became negative to light at about 20 minutes after the treatment. Most of the negative animals stayed at the bottom of the negative side of the beaker. In the mixture of 0.8 c.c. of m-KCl and 50 c.c. of the artificial sea-water, which was the optimum proportion, about 75 per cent. of the larvæ became negative to light at about 30 minutes after the treatment.

The mixture of 15 c.c. of isotonic NaCl and 35 c.c. of the artificial sea-water was also tested. It changed the reaction of about 85 per cent. of the larvæ. In comparing these results with those shown in Table III., it may be concluded that the effective order of ions is variable as other environmental conditions are varied. It is little wonder therefore that the order of ionic actions obtained by different investigators is not always uniform.

6. *Effects of Elimination of Certain Salts from the Artificial Sea-water.*

By eliminating each separate component from the artificial sea-water made up of six components, the specific rôle which such component played on either positive or negative heliotropism of the larvæ might be, the writer thought, in part understood.

First of all, NaCl solution was left out of the artificial sea-water. Larvæ transferred into the mixture without NaCl at 22° C., were practically all motionless immediately after the treatment. Sodium chloride being the essential component of the artificial sea-water as well as of the natural one, such result should be expected, since the osmotic as well as the chemical conditions are profoundly changed.

According to Ewald, however, "leaving NaCl out of artificial sea-water" "increases the negative and diminishes the positive" heliotropism of the nauplii of *Balanus perforatus* which are naturally positive to light. Such was not the case in *Arenicola* larvæ, as just stated.

Elimination of KCl had a different effect. After transferring the larvæ into the artificial KCl-free sea-water at 23° C., practically all showed positive reaction immediately after the treatment, though a few soon became negative. About 20 minutes after the treatment, about 20 per cent. of all larvæ were at the top of the positive side of the beaker, about 30 per cent. at the bottom of the same side, and about 50 per cent. at the bottom of the negative side. If the larvæ collected at the bottom of the beaker farthest from the source of light could be called negative, one sees a puzzle hardly capable of solution. For, as already seen, addition of 2 c.c. of isotonic KCl solution to 50 c.c. of the of the natural sea-water also reversed about 70 per cent. of the larvæ. In other words, either addition or elimination of potassium salt produced a negativating effect on the larvæ. Besides potassium, however, there was another negativating component in the artificial sea-water, namely sodium. In the unbalanced artificial sea-water, sodium ions might exert a negativating effect on the larvæ.

In the calcium-free artificial sea-water at 23° C. all larvæ swam about at the bottom of the beaker. Their activity was much diminished; and about 15 minutes after the treatment a majority of them became almost motionless. $CaCl_2$ in the artificial sea-water as well as in the natural one is therefore essential to the life and activity of the larvæ, although of the five salts it is present in the smallest proportion. It is well known that calcium and magnesium antagonize the toxic action of sodium and potassium. Magnesium alone seemed, however, not to be sufficient in this case.

In the magnesium chloride-free or sulphate-free artificial sea-water at 23° C., immediately after the treatment practically all the larvæ were positive. About 10 minutes after the treatment about 60 per cent. or more of them were positive, about 20 per cent. negative, mostly at the bottom of the negative side of the beaker, and 20 per cent. or less were scattered at the bottom. During 1 to 5 hours after the treatment about 70 per cent. or less were positive, and the rest were at the bottom of the negative side. When both $MgCl_2$ and $MgSO_4$ were left out at 23° C., those larvæ which were at the bottom of the negative side constituted about 40 per cent. or more, whereas they were about 30 per cent. or more with elimination of either $MgCl_2$ or $MgSO_4$ alone. As has already been seen, the addition of magnesium chloride or sulphate to the natural sea-water had no negativating effect on the heliotropism of the larvæ. On the other hand, the addition of sodium or potassium chloride had such effect. The fact that magnesium and calcium chlorides seem to act as a positivating component in the artificial sea-water as well as in the natural sea-water is therefore clear, whereas potassium and sodium chlorides act as negativating ones. In other words, magnesium and calcium chlorides act in antagonizing the negativating effect of sodium or potassium chloride, or of both, although magnesium cannot antagonize the toxic action of the latter salts as calcium chloride can do, since the larvæ could not remain active and live long in calcium-free artificial sea-water.

7. *Effects of Alcohols.*

In narcotising *Arenicola* larvæ, Lillie used many narcotics. Of alcohols, he found that ethyl alcohol had a negativating effect on the heliotropism of the larvæ. It will be seen in Table IV. that all of the alcohols tested by the writer had more or less effect in making the larvæ negative to light. Difficulty was experienced, however, in getting uniform results in different series of experiments when different stocks of alcohols were used; probably this variability was largely due to impurity of reagents.

Just as Loeb has shown with copepods and *Daphnia*, it is obvious from Table IV. that the higher the alcohol in the series the greater negativating effect it had. This point will be considered a little later. With the exception of two cases, the higher the temperature the quicker and larger was the negativating effect produced. That the larvæ in the mixture of methyl or ethyl alcohol and natural sea-water at room temperature (22°) were less negative than at a temperature five degrees lower (17°) is hard to understand.

Acetone and two esters were also tested. The general features of the results obtained with these narcotics were quite similar to

TABLE IV.

EFFECTS OF ALCOHOLS.

Concentration of Alcohol Solution (made with Sea-water).	Temperature.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.		
		10–15 Min.	20–25 Min.	30 Min.
0.03 m. methyl alcohol.	32° C.	65 per cent.		
	22° C. (R.T.)		15 per cent.	
	17° C.			30 per cent.
0.016 m. ethyl alcohol.	32° C.	65 per cent.		
	22° C. (R.T.)		25 per cent.	
	17° C.			30 per cent.
0.002 m. n-propyl alcohol.	32° C.	70 per cent.		
	22° C. (R.T.)		45 per cent.	
	17° C.			35 per cent.
0.0006 m. n-butyl alcohol.	31° C.	70 per cent.		
	21° C. (R.T.)		45 per cent.	
	16° C.			35 per cent.

those obtained with alcohols. In no case, however, were the larvæ normal in behavior when the temperature was lowered either 5° C. or 10° C. below room temperature. On the other hand, production of negative heliotropism at the higher temperature (32°) was much more striking with the esters than with any of the alcohols. Table V. gives a summary of these series of experiments.

TABLE V.

EFFECTS OF ACETONE AND ESTERS.

Concentration of Narcotic Made with Sea-water.	Temperature.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.	
		10 Min.	20 Min.
0.01 m. acetone.	32° C.	40 per cent.	
	22° C. (R.T.)		25 per cent.
0.0045 m. ethyl acetate.	32° C.	90 per cent.	
	22° C. (R.T.)		30 per cent.
0.0025 m. ethyl acetate.	32° C.	100 per cent.	
	22° C. (R.T.)		45 per cent.

From Tables IV. and V. nothing certain can be concluded as to the temperature coefficient in the production of negative heliotropism, although the time needed to reach the maximum point is about halved by a rise of 10° C.

8. *Effect of Saponin.*

A solution of saponin made by adding 0.8 c.c. of 0.1 per cent. saponin to 50 c.c. of natural sea-water was also tried. The influence of temperature was well marked. At room temperature about 10 per cent. of the larvæ became negative after 30–40 minutes in this solution: at 10° above room temperature about 90 per cent. became negative within 20 minutes.

9. *Effects of Fatty Acids.*

"Loeb hat . . . quantitative Versuche über die zur Erzeugung von positivem Heliotropismus bei Daphnien und Copepoden nötige Konzentration verschiedener Säuren und Alkohole gemacht. Die folgende Tabelle gibt die minimale Konzentration für die Erregung von positivem Heliotropismus bei Copepoden für verschiedene Säuren und Alkohole:

Ameisensäure	0.006	N.
Essigsäure	0.006	"
Propionsäure	0.005	"
Buttersäure	0.004	"
Valeriansäure	0.004	"
Capronsäure	0.0019	"
Aethylalkohol	0.19	"
Propylalkohol	0.054	"
Normaler Butylalkohol	0.019	"
Amylalkohol	0.011	"

Man sieht, dass die Wirksamkeit der Alkohole sehr rasch mit steigender Zahl der Kohlenstoffatome zunimmt, während bei den Säuren der Zuwachs nur eben merklich ist. Die Folge ist, dass, während die niedrigen Alkohole viel weniger wirksam sind als die korrespondierenden Fettsäuren, bei den höheren Gliedern dieser Unterschied verschwindet."[1]

In comparing the results obtained by Loeb with those which are given in Tables IV. and VI. a general agreement will be found. It is also worth mentioning that the reversal of the larvæ in acidified sea-water took place immediately after the treatment at a temperature 10° C. higher than room temperature, whereas it took place about two minutes after the treatment at room temperature. In this experiment and others shown in

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[1] Loeb, Jacques, "Handbuch d. Vergl. Physiol.," Bd. IV., Erste Hälfte, S. 470.

Table VI., the temperature coefficient (Q_{10}) of the maximum reversal of the normal heliotropism appeared to be between 2 and 3. The reversal may, therefore, be interpreted as the result of a chemical reaction. The marked physiological activity of the fatty acids seems to depend largely on their lipoid-solubility. At lowered temperatures their effectiveness in producing negative heliotropism is somewhat lessened, as Table VI. shows.

Table VI.

Effects of Fatty Acids.

Concentration of Fatty Acid Solution (Made with Sea-water).	Temperature, Degrees C.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.		
		5 Min.	10–15 Min.	20–30 Min.
0.0025 m. formic acid.	32	90 per cent.		
	22 (R.T.)		85 per cent.	
	12			20 per cent.
0.0010 m. acetic acid.	32	95 per cent.		
	22 (R.T.)		90 per cent.	
	12			30 per cent.
0.0015 m. propionic acid.	32	95 per cent.		
	22 (R.T.)		90 per cent.	
	12			60 per cent.
0.0011 m. butyric acid.	32	95 per cent.		
	22 (R.T.)		95 per cent.	
	12			90 per cent.
0.00075 m. valeric acid.	31½	95 per cent.		
	21½ (R.T.)		95 per cent.	
	11½			20 per cent.
0.00006 m. caproic acid.	31½	95 per cent.		
	21½ (R.T.)		95 per cent.	
	11½			

Of all chemicals tested by the writer, fatty acids were the best for reversing normal heliotropism. If one reversed the heliotropism of a mass of larvæ by fatty acid, a large percentage gradually accumulated at the side of the dish away from the light. If now one turned the dish through 180°, it was most striking to see the hosts of larvæ quickly assume the new orientation and swim to the negative side. Their general behavior and activity appeared normal. Many, however, swam at the bottom of the negative side of the dish, especially in solutions of the higher fatty acids. Curiously enough, this efficiency of fatty acids as a reverser of heliotropism runs closely parallel to their physiological efficiency in producing artificial parthenogenesis, as discovered by Loeb.

10. "*Antagonism*" *Between Acid and Narcotics.*

It is known through the work of R. Lillie that an antagonism between salts and narcotics is readily demonstrated in *Arenicola* larvæ. In the present investigation it was found that chloroform, ethyl alcohol and ether had little or no negativating effect on the larvæ at room temperature; on the other hand, butyric acid proved to be the most effective of all the negativating agents employed. The writer thought therefore that antagonism might be demonstrated in the mixture of any of those narcotics and butyric acid. It was found that the negativating effect of butyric acid was much retarded in the presence of a narcotic. In sea-water containing .0022 m. butyric acid 95 per cent. of the larvæ became negative after 15 minutes exposure. In the same solution to which chloroform, ether, or formaldehyde were added in appropriate concentrations no reversal was evident after 15 minutes and only 50 per cent. were negative after one hour's exposure, as compared with 90–100 per cent. in the control. Apparently the process of reversal is retarded in the presence of the anæsthetic.

11. *Effect of Solutions of Butyric Acid made with Artificial Sea-water.*

The writer tested the effect of butyric acid in artificial sea-water. It was found that a very weak concentration of butyric acid, *i. e.*, 0.00006 mol., was efficient in negativating about 80 per cent. of the larvæ in about 10 minutes after the treatment.

12. *Effects of Inorganic Acids.*

Loeb[1] found that the monobasic fatty acids were very effective, whereas strong acids, such as HCl, HNO_3 and H_2SO_4 "had so little effect as to be practically useless" for experiments on the membrane formation of sea-urchin eggs in artificial parthenogenesis. He concludes that this proves a diffusion of undissociated molecules into cells and denies an increased permeability of the cells for hydrogen ions.[2] The writer tested this idea in producing the negative heliotropism of larvæ, but the results obtained by the writer did not agree well with those of Loeb, as in the case of fatty acids. For instance, in 0.0036 m. HCl solution made with sea-water, about 80 per cent. of the larvæ became negative and in similar solutions of H_2SO_4 about 95 per cent. became negative 15 minutes after the treatment at 21° C. The negative larvæ, however, showed marked abnormality. This was the only case of failure, so far as the writer found, in the parallelism between the effects of chemical substances in producing artificial parthenogenesis in sea-urchin eggs and in reversing the positive heliotropism of *Arenicola* larvæ.

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[1] Loeb, Jacques, *Biochem. Zeits.*, Bd. 15, S. 254, 1909; "Artificial Parthenogenesis and Fertilization," p. 133, 1913.

[2] Loeb, J., *loc. cit.*, pp. 142–143.

The writer also found at the Marine Biological Laboratory of the Kyushu Imperial University, Fukuoka, Japan, in the summer of 1916, that a marine crustacean, *Astracoda Cypridina* (*hilgendorfi*) which was strongly negative to light, could be made positive by HCl or H_2SO_4 solution. Quantitative data were not obtained owing to illness.

That acids, organic and inorganic, are able to penetrate the living cell, is proved by Harvey.[3] It seems possible therefore that both HCl and H_2SO_4 penetrated into *Arenicola* larvæ.

13. *Effects of Strong and Weak Bases.*

Loeb discovered that "the weak base NH_4OH is much more efficient for the causation of artificial parthenogenesis in *Arbacia* eggs than the strong bases, NaOH, KOH and tetraethylammonium hydroxide,"[1] $N(C_2H_5)_4OH$. The writer found that this was also the case in the production of negative heliotropism in *Arenicola* larvæ. The essential results of his experiments may be summarized thus: in the appropriate concentration of NH_4OH, about 70 per cent. of the larvæ became negative after about 20 minutes exposure to the solution at 21° C., whereas in equivalent solutions of NaOH, KOH, or $N(C_2H_5)_4OH$, only about 10 or 15 per cent. of the larvæ became negative after an exposure of about 60 minutes at this temperature. This difference may be explained by the fact that the living cell is permeable to ammonium hydroxide but impermeable to sodium hydroxide and other strong bases, as experiments of Bethe[2] and Warburg[3] have clearly shown. Possibly the weak base NH_4OH is more effective than the stronger bases because of greater solubility in the lipoids of the plasma membranes.

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[1] Harvey, E. Newton, *Science*, N. S., Vol. 39, p. 947, 1914.

[2] Loeb, Jacques, *Jour. Ex. Zoöl.*, Vol. 13, p. 577, 1912.

[3] Bethe, Albrecht, *Pflüger's Arch.*, Bd. 127, S. 219, 1909.

[4] Warburg, Otto, *Zeits. physiol. Chem.*, Bd. 66, S. 305, 1910.

14. *Effects of Chemicals after Returning into Normal Sea-water.*

Lillie observed one curious effect in larvæ treated with one fourth and one fifth saturated solutions of chloroform in sea water; when the larvæ, after about two hours in corked flasks containing these solutions, were poured out into watch glasses the great majority were found, after three or four hours (when the chloroform had evaporated), to exhibit a pronounced negative phototaxis in place of the usual positive. . . .[1] As was pointed out in his previous paper, the writer found a similar phenomenon in the larvæ treated with sodium, potassium, calcium and magnesium chloride solutions when they were returned into normal sea-water.[2] This was particularly remarkable in the larvæ treated with the last two solutions during the actual immersion in which there was never any reversal of positive heliotropism.

In the present experiments, the same phenomenon was also found in larvæ treated with salts, narcotics—chloroform, chloral and formalin in particular—strong alkalies, potassium cyanide, etc. As already shown, most of these chemicals were not favorable agents for the reversal of the positive heliotropism of the larvæ. Moreover, in some cases, KCN, for instance, the larvæ remained positive or indifferent to light while in presence of the chemical substance (dissolved in normal sea-water). Nevertheless the "after-effect" was pronounced. That is to say, almost all larvæ became negative when returned to normal sea-water.

Such facts are consistent with the hypothesis that these chemicals act by changing in some way the normal properties of the plasma membranes of the irritable elements concerned, so that a certain time is required to recover normal conditions after return to normal sea-water.

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[1] Lillie, R. S., *loc. cit.*, p. 34.

[2] Kanda, S., *loc. cit.*

[3] Lillie, R. S., *loc. cit.*, p. 34.

IV. SUMMARY.

1. At room temperature nearly all *Arenicola* larvæ in the early swarming stage in normal sea-water are positively heliotropic. At a temperature 10° C. higher (30°–34°) about half of the larvæ become negative to light after about 5 minutes' exposure. At 10° C. lower than room temperature (ca. 12°), about 20 per cent. of the larvæ become negative after about 25 minutes exposure.

2. In sea-water made hypertonic by addition of NaCl or KCl some larvæ (20–35 per cent.) become negative.

3. In sea-water made hypertonic with $CaCl_2$, $MgCl_2$ or $MgSO_4$, no reversal was observed.

4. In hypotonic sea-water, about 90 per cent. of the larvæ reversed their heliotropism; reversal takes place more rapidly the higher the temperature (between 12° and 32°).

5. Isotonic solutions of ammonium, potassium, lithium, sodium chlorides and sulphates made in sea-water reverse the positive heliotropism of the larvæ. But isotonic solutions of magnesium and calcium chlorides and sulphates in sea-water do not. The reversing effect of these salts seems, therefore, due to the cations. This suggests an action on the plasma membranes (*cf.* the membrane theory of Ostwald-Bernstein).[1] The effective order of these cations is

$$Na^+ < Li^+ < K^+ < NH_4^+$$

6. In artificial sea-water, the positive heliotropism of the larvæ is not noticeably different from that in natural sea-water. A trace of alkali makes apparently no difference.

7. Hypertonic sodium and potassium chloride solutions added to the artificial sea-water reversed the positive heliotropism of the majority of the larvæ.

8. In sodium-free artificial sea-water, the larvæ became motionless immediately after the treatment (effect of hypotony).

9. In potassium-free artificial sea-water, many became negative to light, and remained at the bottom of the negative side of the beaker.

10. In calcium-free artificial sea-water, the larvæ became motionless about 15 minutes after the exposure. Magnesium

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[1] Bernstein, J., *Pflüger's Arch.*, Bd. 122, S. 129.

alone, therefore, cannot antagonize the toxic action of sodium and potassium salts.

11. In the magnesium-free artificial sea-water, many remain positive to light. This fact may be explained as an antagonistic action of calcium toward sodium and potassium salts which acting by themselves are negativating agents.

12. Alcohols (methyl, ethyl, n-propyl, n-butyl) as used by the writer are not favorable as negativating agents at room temperature, though they are good at higher temperature. The higher the alcohol in the series the greater negativating effect it has.

13. Most of the larvæ treated with esters, methyl and ethyl acetate, became negative at higher than room temperature.

14. Other compounds, chloroform, formalin, ether and saponin, produced little or no reversal while the larvæ were in the solutions, except saponin at higher temperatures.

15. Monobasic fatty acids are the best of all chemicals used for this work. The higher the acid in the series the greater negativating effect it has.

16. Some narcotics "antagonize" or retard the reversing action of butyric acid.

17. The reversing effect of a very weak concentration of butyric acid (.00006 m.) in artificial sea-water is great.

18. Hydrochloric and sulphuric acids produce negative heliotropism, almost as well as fatty acids do.

19. In producing negative heliotropism, NH_4OH is much more efficient than strong bases.

20. *Arenicola* larvæ treated with narcotics become negative after returning into normal sea-water.

THE RELATION OF PLUMAGE TO OVARIAN CONDITION IN A BARRED PLYMOUTH ROCK PULLET.[1]

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The assumption by female birds of plumage and other external characters which ordinarily distinguish the male is not an uncommon phenomenon and has long been noted. While it is known to occur among wild birds (Hunter, 1780, p. 532), it has been much more commonly observed in domesticated varieties or others retained in captivity, where the opportunity for close observation is obviously much greater. An excellent general summary of the literature on the subject, including a most useful bibliography, has recently been published by Larcher (1916).

Cases have most frequently been reported in fowls, pheasants and ducks, and it is probable that most poultrymen of wide experience have known of their occurrence. A careful search of the poultry journals would doubtless reveal mention of many cases, though the proportion which gets into print must be relatively small, and the number that receive scientific attention even smaller. One such has been reported recently (Dent, 1917) of a mongrel Barred Plymouth Rock which was claimed not only to have laid eggs but also to have treaded hens. It was stated, furthermore, that the eggs from these hens with which the hermaphrodite mated were successfully hatched. The bird in question was also said to crow at times and to sing like a hen every day. A photograph accompanying the letter shows a bird with a head like a cock, but body more resembling a hen. If confirmed, this would be an extreme case of hermaphroditism.

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[1] Contribution from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 14, and from the Kansas Agricultural Experiment Station. Published with the approval of the Directors.

An extensive study of a number of similar, though less extreme cases, has just appeared (Boring and Pearl, 1918), and these will be mentioned later in connection with our own case.

In the foregoing cases the non-functioning of the ovary and the consequent deficiency of ovarian secretion was due to natural causes, probably in most instances to a diseased condition of the ovary. The deficiency of ovarian secretion can also be brought about by artificial removal of the ovary. This is a delicate operation but has been successfully accomplished and the effects on ovariotomized birds have been reported by Guthrie (1910) on a hen, Fitzsimons (1912) on ostriches, and Goodale (1913, 1916*a*, 1916*b*) on chickens and ducks. In all cases more or less complete assumption of characteristic male plumage, and in varying degrees of other masculine characters, appeared after the ovaries were removed, some individuals, as Goodale says, becoming "nearly complete replicas of the male."

The subject of the present note was a purebred Barred Plymouth Rock pullet. She was hatched on the poultry farm of the Kansas State Agricultural College in 1915 and received the number 1050, as a consequence of which she has since been designated in our records as "Kansas 1050." In the early months of her life she was not noticeably different from her sisters and flock mates, and it therefore happens that no photograph was taken of her during that time. In February, 1916, she was put into a breeding pen as a normal pullet, along with others, but some time later it was noticed that she was taking on marked male characteristics, particularly with respect to plumage. Fig. 1 shows her appearance in April, 1916, while Fig. 2 is one of her normal flock sisters at about the same time. For purposes of comparison a photograph (Fig. 3) is also introduced of a normal cockerel, a full brother of the pullet shown in Fig. 2. A comparison of these photographs shows even better than words can describe the character relations of the three individuals. It will be seen that "Kansas 1050" has almost perfect male plumage, except that the sickle feathers of the tail are not so long and the breast and under parts are slightly darker, due to the fact that the white bars on the feathers are somewhat narrower in proportion to the black. The characteristic difference in barring in males and females of the same strain is illustrated in Figs. 2 and 3. This will be discussed later.

The change in plumage between February and April denotes of course, that a molt occurred during this period. As the normal season for molting is in the fall, this is in itself a matter of interest and implies some underlying physiological process. In view of the close relation well known to exist between molting and ovarian activities, it seems reasonable to suppose that the bringing on of the molt at an unusual season, as well as the modification of the character of the new plumage, was due to changes in the ovary induced by the development of the pathological condition to be described later. While the effect on the character of the plumage is very likely the direct result of the action of changed internal secretions from the sex gland, it seems more probable that the molt is a secondary effect due to the change in metabolism, since in general molting seems to be most closely linked to metabolic conditions of the individual. This is indicated by the fact that molting may at times be induced by other conditions than changed sex-gland activity, such as change of feed, injury, and other environmental influences.

In other respects "Kansas 1050" was at that time rather intermediate between the normal pullet and cockerel. The head and comb were more like that of the pullet; shape and carriage of the body were intermediate. Spurs were not developed. In general she may be said to have looked much like a capon, a resemblance which was noted by a number of observers.

"Kansas 1050" was brought to Madison in the fall of 1916 and was merely kept under observation all of that winter. Her behavior during that time showed nothing of special interest, and in so far as indications of sexuality were concerned was practically indifferent. She seemed in excellent health and "sang" a great deal, somewhat resembling a hen in this respect, except that the voice had a peculiar more or less masculine quality. She was never heard to crow. She showed no indications of laying, and it was suspected that she probably had an ovarian tumor, which would account also for the plumage change. Thinking that possibly the accessory sexual organs might be normal even though the ovary was affected, we decided to try the experiment of removing the tumor and of ingrafting an ovary from another individual, in order to see what effect it would have on the secondary sexual characters, and also in the hope that eggs might be produced normally from the engrafted ovary.

The operation was undertaken on April 8, 1917, an incision being made in the usual place for caponizing. This disclosed an enormous tumor, estimated to be some 10 cm. in diameter, and highly vascular on the surface. It seemed probable that the tumor was of ovarian origin, but on account of its size its point of attachment could not be definitely determined. Its removal was, moreover, deemed impracticable because of the highly developed and ramifying vascularization. Consequently several pieces of ovary from a freshly killed "white" Andalusian pullet were inserted loosely into the body cavity, and the wound closed. This pullet was nearly ready to lay, an ovum having already entered the oviduct; but only pieces of the ovary with very small ova were used for transplantation.

The patient made an uneventful recovery, except that the ether used as the anæsthetic apparently caused some irritation of the air passages, resulting in a slight "rattling" in the breathing, which persisted for some time. General health, however, appeared excellent, and the bird was bright and active.

On May 3 a white Wyandotte cock was put into the pen with "Kansas 1050" to test their behavior. He showed no antagonism to "Kansas 1050" but treated her as if she were a female, in spite of her male appearance. She on her part behaved more like a female, showing no fear of the cock and coming at his food call. She did not, however, display any signs of sexual activity.

On this date new feathers were beginning to come in on the area which had been plucked for the operation, and as they seemed to differ somewhat in character from the old feathers, other areas were denuded in order to make a further test of the matter. For this purpose the hackle, saddle and sickle feathers were plucked from the left side of the body, leaving those on the right side in place. By July 28, when the photographs reproduced in Figs. 4 and 5 were taken, the feathers on the neck and lower back had been entirely replaced, but new tail feathers had not yet grown out. Fig. 4 shows distinctly the difference in the old feathers on the right side of the neck and the new feathers on the left side. The difference is even more marked on the lower back, as shown in Fig. 5. In both locations the new feathers on the left appear darker and are distinctly hen-feathers in shape while the older feathers of the right side are lighter and elongated like those of a cock. A careful comparison of the two sets of feathers was made to ascertain how closely they resembled normal feathers of the two sexes respectively.

The American Standard of Perfection[1] stipulates that in the ideal of the Barred Plymouth Rock breed, "the light and dark bars [are] to be of equal width, in number proportionate to length of feathers, and to extend throughout the length of feathers in all sections of the fowl." Every breeder who has had any experience with this breed of fowls knows that this standard is a purely artificial one, for in any particular strain the males always run lighter in color than the females (compare Figs. 2 and 3) there being, as Pearl and Surface (1910, p. 47) remark, "actually a sexual dimorphism in respect to plumage color in Barred Plymouth Rocks as they are ordinarily bred." This has driven the fancier to a system of breeding commonly known as "double mating," but which really means the carrying along side-by-side of what are virtually two sub-varieties of the breed, one in which the pullets are very dark and the cockerels approach the Standard requirements, and the other in which the pullets are about medium and the cockerels much lighter. For showing in the Standard classes males are chosen from the former line and females to match them from the latter. The dark "cockerel bred" pullets and the light "pullet bred" cockerels are sometimes shown in entirely distinct classes as breeding stock. The males and females of Standard color are not bred together, but each is mated to selected birds of its own breeding.

This fact was commented on a number of years ago by Spillman (1909), who suggested it would be wiser for the fancier to endeavor to learn just the shade of difference which "nature tries to produce between the sexes and then change the Standard accordingly." His explanation of the natural difference in shade in the two sexes is that the males are homozygous whereas the females are heterozygous with respect to barring. "The males, therefore, have twice as much tendency to barring as do the females. The males are therefore more barred than the females and hence are lighter in color." By "more barred" Spillman means that the white bars are wider in proportion to the black. This difference in the relative widths of the white and black bands on the feathers may be noted in the normal cockerel and pullet, Figs. 2 and 3, but is more evident in the individual feathers shown in Figs. 7–10. These feathers, while not from the same individuals shown in Figs. 2 and 3, are from birds of the same breeding as these and "Kansas 1050." Fig. 7 is a neck feather from hen 804, which was a flock sister of "Kansas 1050," while Fig. 8 is a feather from the cushion, or lower back, of the same bird. Fig. 9 represents a hackle feather, as it is called in the male, from the neck of a cock bird, 104E, which is the son of a flock sister of "Kansas 1050." Fig. 10 is a saddle feather from the same male, being from the position corresponding to the cushion in the hen. It will be noticed that in addition to the differences in shape in the two sexes, the white and black bars on the hen's feathers are of practically the same width, while on those of the cock the white bars average considerably wider.

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[1] Amer. Poult. Assn., 1915, p. 50.

From the photograph of the neck feathers of "Kansas 1050" in Fig. 4 the impression is gained that the masculine feathers of the right side have broader white bars; but that such is not the case on the lower back is evident from a careful inspection of Fig. 5. Furthermore, the fact that the white bars are of the same width in the old and new feathers of both these regions is convincingly shown in Figs. 11–14. Figs. 11 and 12 are neck and lower back feathers respectively from the left or "feminine" side, while Figs. 13 and 14 are corresponding feathers from the right or "masculine" side. The lighter appearance of the "cock" feathers of the right side is therefore not due in this case to relatively wider white bars, but must be attributed to the fact that they are older and consequently the black has faded to some extent, and to the difference in form and structure. Normal cock feathers are more elongate in these regions, and they differ in structure from the hen feathers in that the firm web is relatively as well as absolutely narrower. In the feathers of the female the barbules interlock practically to the edge of the feather, making nearly the whole surface into a fairly firm web, but in the male the hackle and saddle feathers have a firm web only in the middle portion, practically the outer half[2] of the barbs being free of barbules and presenting a frayed appearance. These free barbs, together with the longer, pointed loose ends of the feathers cause the relatively rough, stringy texture of the hackle of the cock as compared with the smooth neck of the hen. The loose structure of the feathers in the former case, causing greater diffraction of the light, undoubtedly contributes to a lighter appearance of the hackle, irrespective of the broader white bars under normal conditions. Thus it will be noted that in "Kansas 1050," the masculine feathers assumed presumably as a result of the diseased ovarian condition were like cock feathers in respect to shape and structure, but still resembled hen feathers in the matter of width of bars, as shown by comparison with the new hen feathers grown after the new ovarian tissue was introduced.

The above would appear to support Spillman's interpretation of the normally wider white bars in the male as being due to homozygosis for the factor for barring. The form and structure of the feathers would thus be considered as secondary sexual characters dependent on the functioning of the sex gland; barring, however, being dependent directly on the genetic factor, would remain the same, whatever the sexual hormones. Although the failure of the ovary to function properly gave rise to a condition of internal secretion which led to the assumption of male plumage with respect to shape and structure of feathers, the soma was still heterozygous for the barring factor and consequently the white bars in the "cock" feathers remained narrow as in the normal female. Davenport (1912, p. 17) classified sex-dimorphic characters into two classes: "(*a*) characters whose development is controlled primarily by determiners located in the sex-chromosomes and, (*b*) characters whose development is specially influenced or modified, probably by secretions of the sex-glands." In "Kansas 1050" we have both these classes represented in the same feather.

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[2] Goodale's statement (1918, p. 292) that "the secretion of the ovary is necessary for the development of the barbules of the feathers of the dorsal regions since the barbules do not develop along the entire length of the barb in the male or in the capon" seems to be at variance with the above. If he means, as he seems to say in substance, that there are no barbules in the hackle, back, or saddle feathers of the male, his observations are certainly different from ours on Barred Plymouth Rocks. It is true that at the tip of the hackle feathers there are no barbules, but farther down they are to be found at the bases of the barbs, while just above where the fluff begins they run out practically to the extremities of the barbs. The statement that they are free on "the outer half" represents the average condition, or that found about midway from the fluff to the tip of the feather.

In the hackle and saddle feathers of the male the bars are less straight and regular than in the corresponding feathers of the female or in other parts of the plumage of either sex. In general there is a lagging of the middle portion of the bar, at the shaft, producing a more or less V-shaped bar instead of one straight across. This is illustrated in Figs. 9 and 10, and Figs. 13 and 14 show that the corresponding feathers on "Kansas 1050" had the same character. Thus it would seem that *shape* of bar, unlike *width*, is a secondary sexual character rather than sex-linked.

But while our case conforms to the interpretation given above, Pearl and Surface (1910, p. 53) state that in the cross between Barred Plymouth Rock and Cornish Indian Game "there is the same sort of sexual dimorphism in color pattern to be observed in the hybrids as in the pure Barred Plymouth Rocks," and specifically that "the male hybrids have narrower dark bars on the feathers especially in hackle and saddle feathers than do the females." These cross-bred males must of course have been heterozygous for barring, so that on Spillman's hypothesis they should have had the same width of bars as the hens. The fact that the crossbred males were darker than pure Barred Rock males does not alter this point, so that the matter cannot be considered as settled. It is of course possible that there were genetic factors carried by the non-barred breed (Cornish Indian Game) which might have had some modifying effect on the barring of the crossbred offspring.

"Kansas 1050" was killed and autopsied on July 28, 1917. Her behavior subsequent to the operation had not been noticeably different, but it was noted that for two or three weeks prior to the above date she had often been observed forenoons sitting in a nest-like depression in the straw. The notes also state that she had been a consistent "singer," keeping up a little crooning note a good share of the time.

The autopsy revealed the visceral organs normal except for an enormous tumor (Fig. 6) which filled practically all of the left side of the abdominal cavity and caused a marked abdominal protrusion. The tumor was for the most part highly vascular on the outside; it was roughly knobby, the larger knobs being several centimeters in diameter and dark purplish, almost black, in color; most of the smaller ones somewhat more reddish. The freshly removed tumor weighed 289 grams. The bird was not weighed before killed, but after the tumor was removed, most of the blood washed out and nearly all the feathers off, the carcass weighed 2,350 grams.

The tumor was attached at the normal site of the ovary, though the mesenteries were somewhat adherent to it at other points. There appeared to be no normal ovarian tissue remaining, nor, for that matter, was any trace found of the implanted pieces of ovary—at any rate anything which was positively identified as such.

The interior of the large knobs of the tumor exhibited a coarse trabecular structure, the trabeculæ being very dark and the matrix of a yellowish brown color. The smaller, softer knobs contained considerable blood, while the others were firm and hard. Greatest length of tumor, measured fresh, 13 cm.; breadth, 9.5 cm.

The oviduct had the appearance of that in a normal non-laying pullet.

The tumor was preserved and later referred for examination to Dr. C. H. Bunting, of the Department of Pathology at the University of Wisconsin. He has kindly given us the following description based on his study of the specimen. It seems advisable to publish this in full as a contribution to the knowledge of the anatomy of ovarian tumors in fowls.

"The specimen consists of a somewhat cruciform laked mass approximately 12.5 × 8.5 × 7.5 cm. in size. A distal small lake approximately 2.5 cm. in diameter appears to be a subcapsular hæmorrhage. A large central lake appears to be made up almost entirely of large vascular channels and to be the site of extensive hæmorrhage. The proximal lakes show numerous large vascular channels separated by tissue, in the main rather translucent and glistening, but showing numerous small opaque areas.

"*Microscopical Study.*—Sections from the larger lake show many large channels filled with blood. Some of the larger of these are thrombosed; some of the thrombi are comparatively recent, others are old and show deposits of calcium salts in the hyaline material. The intervening tissue, which is small in amount, is necrotic.

"Sections from the proximal lakes show numerous vascular channels, few of which are thrombosed. These channels are separated by a considerable amount of connective tissue, which is in most places cellular, and apparently of recent growth. This tissue is in many places thickly infiltrated with lymphocytes and with numerous cells with eosinophilic granulation. There are recent small hæmorrhages into the interstitial tissue and evidence of old hæmorrhages, in the presence of hæmosiderin masses.

"In addition to the changes described there are throughout the intervascular tissue in these sections alveoli of various sizes, but usually small, solidly filled with cells of an epithelial (or endothelial) appearance. The cells vary much in size and appearance. The average cell has a nucleus of from 12 μ to 15 μ in diameter with distinct nucleolus and fairly heavy chromatin network. The protoplasm is abundant, granular, and shows a tendency to fatty degeneration. The cell outline varies. In some alveoli they are almost columnar, in others spindle-shaped, and in others their outline is difficult to determine as the cells are closely united into sheets or columns resembling a syncytium. While most of the vascular channels are lined by a delicate flattened endothelium, in some places they have a lining of high irregular cells of the character seen in the intervening alveoli. One further gains the impression that it is from such layers that the cells of the alveoli are derived. No evidence of ovarian structure is found in the sections.

"*Diagnosis.*—Vascular malignant tumor, apparently of endothelial origin."

The case of "Kansas 1050" falls naturally into the series described by Boring and Pearl (1918). The assumption of male plumage was obviously due to the degenerate and pathological condition of the ovary, and admits of the same interpretation that they give, namely that the general correspondence of the secondary sex characters to the primary sex organs "might be accounted for in accordance with the theory that the ovary forms an external secretion that inhibits maleness." No indication of a testis nor of testicular tissue was found in "Kansas 1050," though the search for it was not perhaps as careful as it might have been. The principal interest of our case seems to lie in the enormous size of the tumor, associated with apparent good health, the immediate effect on the incoming feathers of implanted ovarian tissue and the relations of form, structure and barring of the two sets of feathers.

SUMMARY.

1. A purebred Barred Plymouth Rock pullet, designated as "Kansas 1050," which at first appeared normal, later assumed plumage much more resembling that of a normal male, and became much like a capon in general appearance.

2. This change in secondary sexual characters was obviously due to the development of a very large ovarian tumor.

3. Implantation of ovarian tissue from a normal pullet produced an immediate effect on new plumage assumed. New feathers grown in the few weeks succeeding the operation were definitely hen feathers.

4. The "male" feathers assumed early in life as a result of the pathological condition of the ovary, though like normal male feathers in shape and structure, resembled hen feathers in respect to barring. This distinguishes clearly the difference between *secondary sexual dimorphism*, as exhibited in the first instance, and *dimorphism caused through sex linkage*, as illustrated by the barring.

BIBLIOGRAPHY.

American Poultry Association

'15 The American Standard of Perfection. Illustrated. A complete description of all recognized varieties of fowls. Revised. 368 pp. Publ. by Amer. Poult. Ass'n.

Boring, Alice M., and Pearl, Raymond.

'18 Sex Studies. XI., Hermaphrodite Birds. Journ. Exper. Zoöl., Vol. 25, No. 1, pp. 1–47, pls. 1–9.

Davenport, C. B.

'12 Sex-limited Inheritance in Poultry. Journ. Exper. Zoöl., Vol. 13, No. 1, pp. 1–18, pls. 1–8.

Dent, O. F.

'17 [Hermaphrodite Fowl.] Reliable Poultry Journal, Vol. 24, No. 3, May, p. 335.

Fitzsimons, F. W.

'12 A Hen Ostrich with Plumage of a Cock. Agric. Journ., Union S. Africa, Vol. 4, pp. 380, 381. (See also Veterinary Journ., Vol 69, No. 459, Sept., 1913, pp. 414, 415.)

Goodale, H. D.

'13 Castration in Relation to the Secondary Sexual Characters of Brown Leghorns. Amer. Nat., Vol. 47, pp. 159–169.

'16a Further Developments in Ovariotomized Fowl. Biol. Bull., Vol. 30, No. 4, pp. 286–293.

'16b Gonadectomy in Relation to the Secondary Sexual Characters of Some Domestic Birds. Carnegie Inst. Wash., Publ. No. 243, 52 pp., pls. 1–7.

'18 Feminized Male Birds. Genetics, Vol. 3, No. 3, pp. 276–299, pls. 1, 2.

Guthrie, C. C.

'10 Survival of Engrafted Tissues. I (A) Ovaries and (B) Testicles. Journ. Exper. Med., Vol. 12, pp. 269–277, pls. 22, 23.

Hunter, John

1780 Account of an Extraordinary Pheasant. Philos. Trans. Roy. Soc. Lond., Vol. 70, Part 1, pp. 527–535.

Larcher, O.

'16 Contribution à l'histoire des femelles d'Oiseaux chez qui se développent les attributs du sexe male. Rec. de Méd. Vétér., T. 92, Nos. 11, 12, pp. 173–183.

Pearl, Raymond, and Surface, Frank M.

'10 On the Inheritance of the Barred Color Pattern in Poultry. Arch. f. Entwicklungsmech. d. Org., Vol. 30, part 1, pp. 45–61, pls. 2, 3.

Spillman, W. S. (should be W. J.)

'09 Barring in Barred Plymouth Rocks. Poultry, Vol. 5, No. 11, Aug., pp. 7, 8.

Note.—While the foregoing paper was in press there came to our attention a paper by Albert Pézard on "Le conditionnement physiologique des charactères sexuels secondaires chez les Oiseaux. Du rôle endocrine des glandes génitales," Bull. Biol. de la France et de la Belgique, T. 52, Fasc. 1 et 2, pp. 1–176. This is a very interesting contribution to the literature of the genital glands in birds as endocrine organs and has an extensive bibliography, mostly of foreign references.

Explanation of Plate I.

Fig. 1. Barred Plymouth Rock pullet "Kansas 1050" after assumption of male plumage.

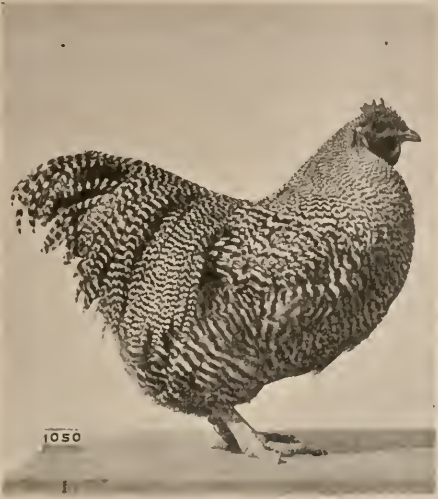
Fig. 2. Normal Barred Plymouth Rock pullet (Kansas Station No. 890), a flock sister of "Kansas 1050," and of approximately the same age.

Fig. 3. Normal Barred Plymouth Rock cockerel (Kansas Station No. 21M), a full blood brother of pullet 890.

Fig. 4. Plumage on back of neck of "Kansas 1050," photographed on July 28, 1917, nearly four months after implantation of ovarian tissue. The difference between the old "cock" feathers on the right side and the new "hen" feathers on the left side is plainly distinguishable.

Fig. 5. Dorsal view of "Kansas 1050," taken on July 28, 1917, showing the old and new plumage, particularly on the lower back.

Fig. 6. Ovarian tumor from "Kansas 1050."



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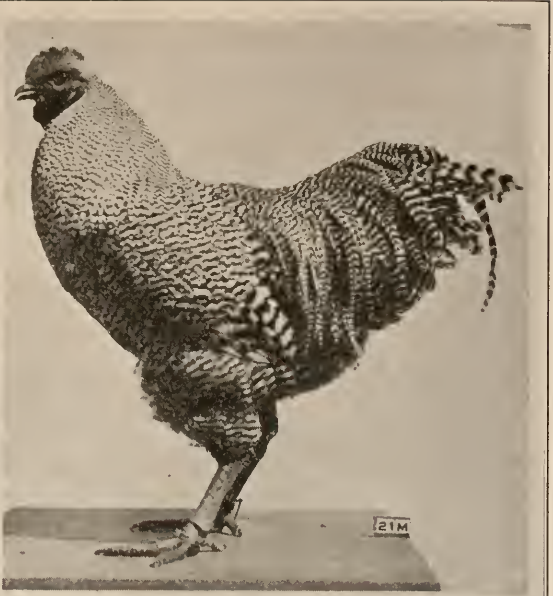

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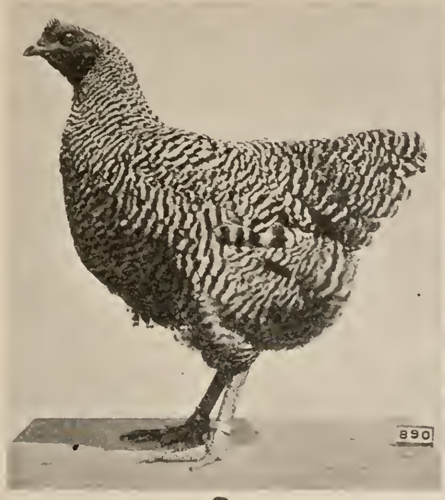

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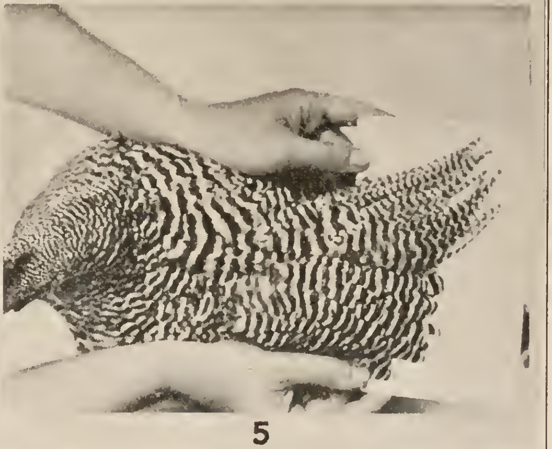

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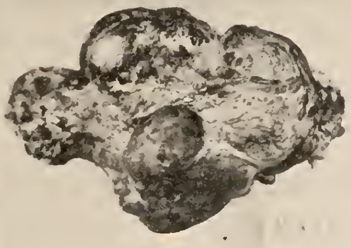

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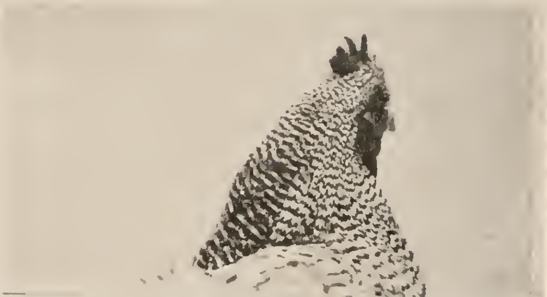

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L. J. COLE AND W. J. LIPPINCOTT.

EXPLANATION OF PLATE II.

Barred Plymouth Rock Feathers.

FIG. 7. Neck feather from normal female (Kansas Station No. 804).

FIG. 8. Cushion feather from normal female (Kansas Station, No. 804).

FIG. 9. Hackle feather from normal male (Kansas Station No. 104E).

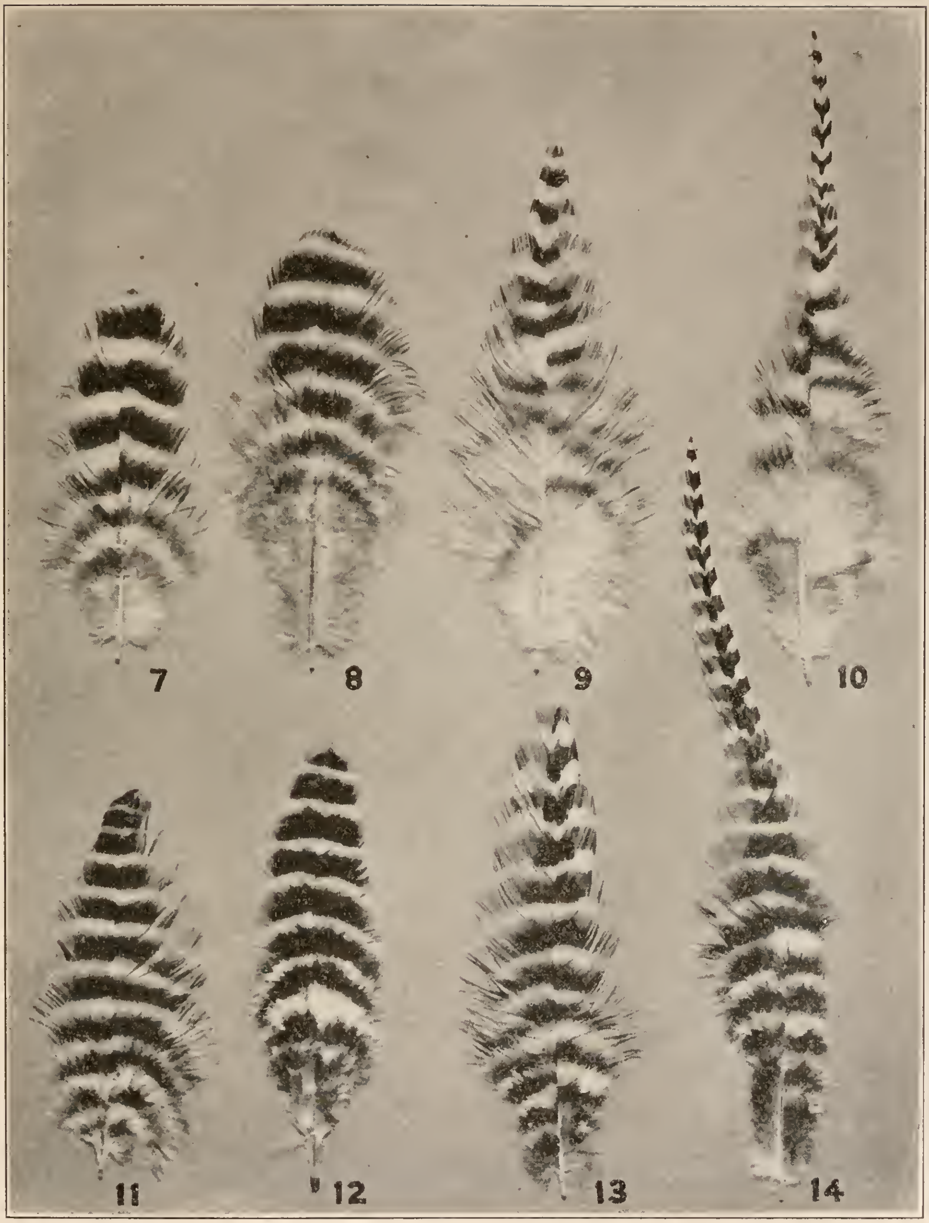
FIG. 10. Saddle feather from normal male (Kansas Station No. 104E).

FIG. 11. Neck feather from left ("hen feathered") side of "Kansas 1050," July 28, 1917.

FIG. 12. Cushion feather from left ("hen feathered") side of "Kansas 1050," July 28, 1917.

FIG. 13. Neck (hackle) feather from right ("cock feathered") side of "Kansas 1050," July 28, 1917.

FIG. 14. Saddle feather (from position corresponding to cushion) from right ("cock feathered") side of "Kansas 1050," July 28, 1917.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L. J. COLE AND W. A. LIPPINCOTT.

THE AXIAL GRADIENTS IN HYDROZOA.

I. Hydra.

C. M. CHILD AND L. H. HYMAN,

Department of Zoölogy, University of Chicago.

In the development and formulation of the general conception of the axial gradient conditions observed or experimentally induced in *Tubularia* and other Hydrozoa have played a part (Child, '15*c*, pp. 79, 91–2, 96–99, 128–137), but particularly during the last five years data concerning the gradient in these forms have been accumulating until now a point has been reached where the volume and variety of these data warrant their presentation. While some of these data have been briefly considered, as noted above and in other papers, most of them have not as yet been recorded, and it is proposed to bring them together in connected form in this and two or three following papers. In the present paper the data on three species of *Hydra* are presented.

Methods and Material.

The gradients in *Hydra* have been studied chiefly by means of the direct or lethal susceptibility method[1] with cyanide in concentrations ranging from *m*/1,000 to *m*/10, ethyl alcohol 1–5 per cent., ethyl ether 1–3 per cent. and the dyes, neutral red, methylene blue and Janus green in wide ranges of concentration. With these agents the regional differences in susceptibility were determined, and the course of the gradient of disintegration and death in body and tentacles was observed under various conditions. Potassium permanganate, which has been found useful in many cases as a means of demonstrating the gradients as color gradients, has also been used to some extent (Child, '19), but is rather unsatisfactory because of the extreme contraction produced.

Observations have been made on the three common species of

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[1] Child, '13*a*, *b*, '14*b*, '15*a*, *b*, chap. III., '15*c*, '16*a*, *b*, *c*, '17, Hyman, '16, 17.

Hydra, *H. viridissima* (*viridis*), *H. vulgaris* (*grisea*) and *H. oligactis* (*fusca*) and data on several hundred individuals have been recorded. The stages used include full grown asexual animals with and without buds, and all stages in the development of buds, and a few observations have been made on sexual individuals, animals developed from the regulation of pieces, and animals reduced in size by starvation.

The observations on *H. oligactis*, begun in 1913 by Child, have been repeated several times since on different stocks of animals and have been confirmed during the present year by Hyman. The observations on *H. viridissima* and *H. vulgaris* were first made by Hyman during 1918 and have been confirmed by Child. All important points concerning the gradients in the three species rest on observations made independently by both of us.

. For the work on susceptibility, Syracuse dishes with covers of very thin glass are used. The animals are placed in the dish in a little water, or in certain experiments most of the water previously present is removed with as little disturbance as possible, the agent, freshly made up to the desired concentration, is added at once, or allowed to flow gently in from a pipette until the dish is quite full, and the dish is then covered with exclusion of all air bubbles. This procedure prevents loss by volatilization and makes it possible to examine the animals at all stages under a low power of the compound microscope, and the dish can be moved if some care is exercised, without disturbing the animals sufficiently to produce contraction.

STRUCTURAL AND FUNCTIONAL SPECIALIZATION OF BODY REGIONS IN THE THREE SPECIES.

The four parts, tentacles, hypostome, column and foot present of course the same general characteristics in all three species, but there are certain features of the different body regions and certain minor differences between the species which are significant in relation to susceptibility.

In the tentacles of all three species the capacity for elongation and contraction apparently decreases basipetally, but the tentacles of *H. oligactis* in the stocks with which we have worked, are much longer and capable of a much greater degree of extension and contraction than those of *H. viridissima* and *H. vulgaris*, which are much alike. The chief differences, however, are in the column. In all three species the basal region of the column including a third to a half the body length in ordinary degrees of extension is more extensile and contractile than the distal half or two thirds. In other words, all three species show at least a functional specialization of a more highly contractile basal region and a less highly contractile digestive region. Since this specialization is particularly significant in relation to susceptibility it will be convenient for present purposes to distinguish the basal region as the "stalk" from the digestive region or "body" in the stricter sense.

In *H. viridissima* this specialization is not usually accompanied by any marked degree of structural differentiation. The column of the fully extended animal is cylindrical and of practically equal diameter throughout its length (Fig. 1),[1] although there is usually a graded decrease in both the amount of nutritive substances and food reserves in the entodermal cells and of the symbiotic algæ toward the base of the column and consequently an increase in transparency in the same direction. In the various stages of contraction this basal region usually undergoes a greater degree of shortening than the more apical regions but is not marked off by any characteristic difference in form from the rest of the column (Figs. 2, 3). In extreme contraction such as is induced for example by strong chemical stimuli, the column may become spherical, or nearly so (Fig. 3).

In *H. vulgaris* a somewhat greater degree of differentiation of the stalk usually appears. In fully extended animals in good nutritive condition, the basal third more or less of the column is distinguishable not only by its thinner wall and greater transparency, the transition from the body to stalk being more abrupt than in *H. viridissima*, but the stalk is somewhat more slender than the body which often shows a slight increase in diameter basipetally to the beginning of the stalk region (Fig. 4). In a moderately contracted condition the stalk is likewise usually

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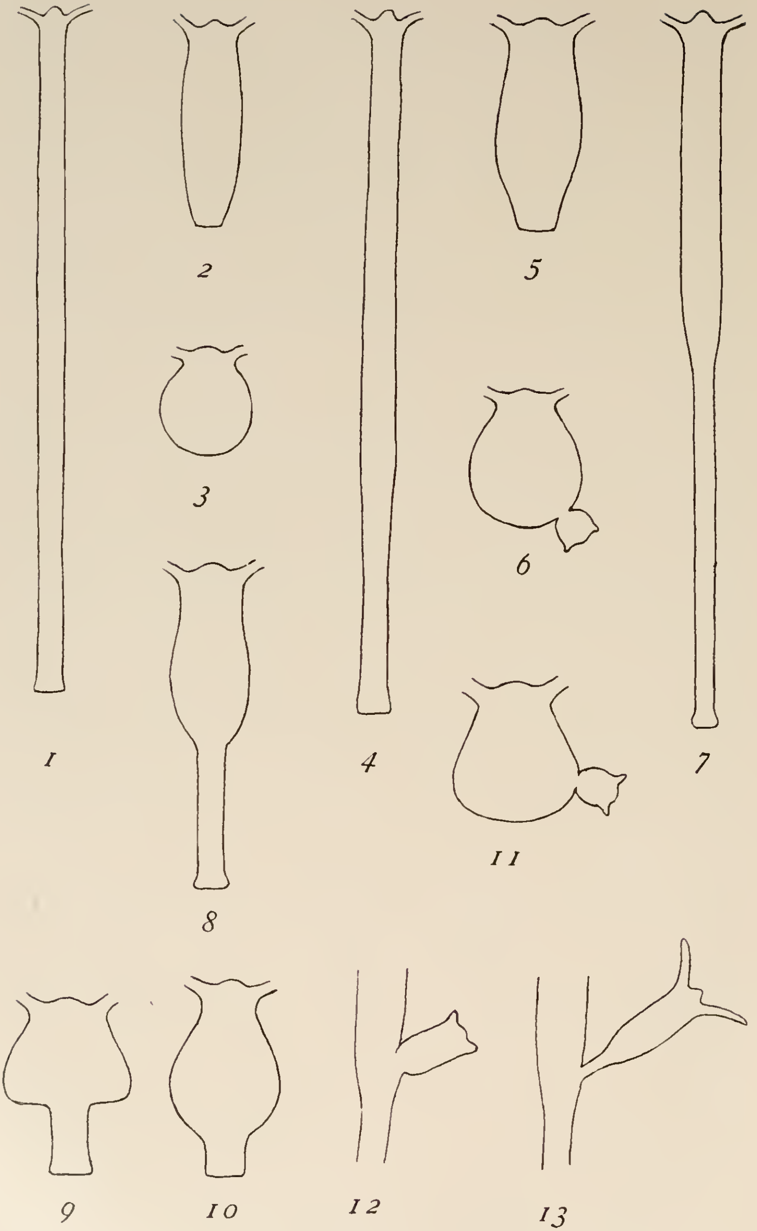
[1] *H. viridissima* is of course much smaller than *H. vulgaris* and *H. oligactis*, but for conveneince in comparison all figures are drawn to such scale that corresponding stages of development of the three species are approximately of the same size.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Figs. 1–13.

distinguishable as a region of smaller diameter than the body (Fig. 5) and in extreme contraction the body is not spherical but more or less urn-shaped with a somewhat flattened base, which represents the contracted stalk. Buds in *H. vulgaris* arise at or near the base of the body proper, and in the state of extreme contraction the position of the bud is at or near the border of the flattened base as indicated in Fig. 6. Since the bud serves as a landmark indicating approximately the boundary between body and stalk its position in Fig. 6 shows that there is in this contracted state a relatively greater decrease in length of stalk than of body.

Hydra oligactis shows a greater degree of differentiation of stalk and body than the other two species. In well extended animals in good nutritive condition the column is very distinctly marked off into two regions, the body proper, including the apical half or less of the column, with a larger diameter and a thick opaque entoderm, and the stalk, comprising the basal half or more of the column, with a smaller diameter very commonly only about half that of the body and a thin, highly transparent entoderm (Fig. 7). Moreover, the transition from body to stalk is in this form much more abrupt than in the other species. Fig. 8 shows a condition of moderate contraction, Figs. 9 and 10 greater degrees of contraction and Fig. 11 a state of extreme contraction induced by chemical stimulation. Since in *H. oligactis* as in *H. vulgaris* buds arise at or near the base of the body, the position of the bud in Fig. 11 indicates approximately the boundary between body and stalk and shows the greater contractility of the stalk.

The differences in diameter, general appearance and opacity between body and stalk become less marked with lack of food and in advanced starvation may almost or entirely disappear even in *H. oligactis*, and with less extreme starvation in *H. vulgaris*. On the other hand, individuals of *H. vulgaris* are not infrequently seen in which the entoderm of the digestive region is so thickened, apparently by nutritive reserves, that the form approaches that of *H. oligactis*, and occasionally *H. viridissima* shows some modification in this direction. But while the shape of the animal may vary widely with nutritive conditions, the differences in specialization between stalk and body appear in general to be greater in *H. oligactis* than in the other two species.

Whatever its degree of development in full-grown individuals, the stalk is not present in young buds (Figs. 12, 14) nor in the earlier stages of the regulatory development of pieces of any of the three species, but is a secondary outgrowth from, or modification of the basal region of the column arising comparatively late in the development of buds (Fig. 13) and of pieces undergoing regulation. Not infrequently buds become detached from the parent before any considerable development of the stalk occurs, and in such cases the stalk develops later in the young, independently attached individual. In its early stages the stalk is very short, much shorter than the body and is not appreciably more contractile than other regions but both its length and its contractility increase until it is commonly somewhat longer than the body in well-extended animals of *H. oligactis* (Fig. 7) and shortens more than the body in extreme contraction (Figs. 10, 11).

The stalk is evidently then a more or less specialized region or organ arising at a late stage of development and functioning in connection with the sessile habit as an organ of extension and contraction. This being the case, it is also evident that the process of budding in hydra takes place at or near the basal end of the body proper, and in this connection it may be suggested that, while the specialization of the stalk is not so great as to prevent its dedifferentiation and regulatory development into a complete individual when it is physically isolated from the parent body, yet it is so much greater than that of the basal region of the body that the buds arise from the latter region rather than from the basal end or any other level of the stalk.

As regards behavior, both the tentacle and the column represent to a certain degree a relation of dominance and subordination. Under natural conditions the more distal regions of the tentacles must be more frequently stimulated than regions near the base, consequently contraction of the basal region of the tentacle is more likely to occur as the result of stimulation of a region distal to it than as the result of direct stimulation from without. While no extensive investigation of the problem has been undertaken simple experiments on mechanical stimulation with

H. oligactis indicate that the tentacle represents to some degree a gradient in physiological condition. While the reactivity of the tentacle varies and exact control of intensity of stimulation is not possible, the sensitiveness of the tentacle seems to decrease somewhat basipetally. Moreover, adequate stimuli near the tip usually induce contraction over some considerable portion or even the whole length of the tentacle, the contraction progressing basipetally, while similar stimuli near the base commonly induce merely a local contraction or bending of the tentacle at the region of contact with the concavity toward the exciting object. A more intense stimulation of the basal region may of course bring about contraction of the whole tentacle, but conduction of the excitation apparently occurs more readily basipetally than in the opposite direction.

Stimulation of one tentacle, if slight, may induce partial or total contraction of that tentacle only, if more intense, of all tentacles and contraction of all tentacles is usually followed by more or less contraction of the column.

As regards the column, contraction of the stalk usually occurs under normal conditions in response to excitation conducted from the tentacles or from some more apical level of the body. Certain degrees of stimulation produce contraction of only the more apical regions of the body or of the body and not the stalk, while more intense stimulation brings about contraction of both body and stalk. In animals partially anesthetized by alcohol, ether and other agents the decrement in conduction apparently increases, even when the body contracts vigorously, and the contraction can be seen to die out before reaching the stalk or in the anterior portion of the stalk. In such cases the stalk may become inactive before the body, not because it has lost its contractility, but because the excitation fails to reach it.

Both body and stalk are much less sensitive than the tentacles to direct contact stimulation. Contraction of the whole column can be induced by adequate stimulation at any point, as in the case of the tentacle, but stimulation of the more apical levels of the body induces, according to its intensity, contraction of only the more apical body region, of the whole body or of body and stalk. Here, as in the tentacle, a decrement in the transmitted excitation evidently exists.

The stalk, with the exception of the foot region, is under normal conditions less sensitive to direct mechanical stimulation than the body. It is often possible to puncture the stalk repeatedly with a needle, without producing any effect beyond a slight local contraction, or at the most some contraction of the whole stalk, but when the stimulus is adequate to produce more than a local effect, contraction of the whole animal usually follows. It appears then that because of the greater sensitiveness of more apical levels and probably also because of greater conductivity in the basipetal direction the physiological state at any level, so far as it is dependent upon excitation, is determined to a greater degree by the conditions in levels apical to it than by conditions at more basal levels. In other words the more apical levels are relatively dominant.

It should be noted, however, that the foot of the attached animal is rather sensitive to direct mechanical stimulation which deforms or stretches it, and the stalk likewise is sensitive to tension, whether produced by currents of water or through the tentacles, and after such stimulation of the foot or stalk, contraction of the whole animal usually occurs. Moreover, the stalk and foot of animals which have been forcibly detached are evidently in a different physiological condition from those of attached individuals, for the stalk of such animals performs searching movements to a greater or less extent, and the foot responds to contact by attachment except in conditions of extreme irritation. The stalk and foot apparently constitute a more or less specialized sensory and motor apparatus which under the usual conditions, *i. e.*, in attached individuals, is to some extent under the control of more apical regions, but which under certain conditions may respond independently or initiate the response of other parts.

THE AXIAL SUSCEPTIBILITY RELATIONS UNDER VARIOUS CONDITIONS.

It is impossible to proceed far in the analysis of the susceptibility relations of hydra without becoming convinced that the contractile activity of any particular region is a factor in determining its susceptibility. The very direct and considerable influence of muscular contraction upon susceptibility is undoubtedly associated with the fact that the muscles of hydra do not constitute a special tissue, but are merely specially differentiated portions of the body cells or of certain of them. This being the case, there is every reason to believe, either that muscular activity in hydra is merely one expression of a general excitation of the cells concerned, or that excitation of the contractile portion of the cell brings about changes in the physiological condition of the cell as a whole, or it may be that both of these possibilities are realized under different conditions.

Whatever the nature of this relation between muscular activity and susceptibility it is necessary in analyzing the susceptibility relations to take into account the condition and activity of the muscular system. It is desirable to determine the susceptibility relations in the body, not only before the muscular system has developed, but also in fully developed animals under conditions of quiescence, moderate and extreme muscular activity and with experimental inhibition of muscular activity. Fortunately it is possible to investigate and analyze more or less completely all these different cases. Although the earlier embryonic stages are not available for work of this kind, the earlier stages of bud-development show little or no muscular differentiation and are to be regarded as essentially embryonic stages. Animals which have been attached by the foot and undisturbed for an hour or more are usually quiescent, while detached animals show a much greater degree of muscular activity. Moreover, different agents and different concentrations of the same agent produce very different effects upon muscular activity before death. Cyanide, for example, except in very high concentrations produces no appreciable excitation at any time before death and, on the other hand, inhibits muscular activity very gradually; it therefore affords favorable conditions for the analysis of the differences in susceptibility in non-motile buds and full grown motile animals and between attached quiescent, and detached animals.

All the other agents used produce more or less excitation accompanied by increased muscular activity in the earlier stages of their action. This condition of excitation may continue for hours in the lower concentrations of these agents, but is of course sooner or later followed by depression, disintegration and death. With these agents then we have a combination of excitation and depression and in consequence of the differences in susceptibility, the degree of excitation and the length of the excitation period differ in different regions of the animals. Again, the degree of excitation and the length of the excitation period vary with concentration of the agent. In the higher concentrations of anesthetics, ethyl ether for example, and to a lesser degree ethyl alcohol, momentary excitation may be followed almost immediately by anesthesia and paralysis of the muscular system.

The dyes used, Janus green, methylene blue and neutral red, are all distinctly excitatory in action, Janus green being an intense irritant, even in very low concentrations, *e. g.*, 0.002 per cent., while methylene blue and neutral red are less irritating in their action. In general the higher the concentration of the dyes in solution, the more intense the irritant action, but in low concentrations the excitation increases to some extent with the accumulation of the dye in the tissues.

With this wide range of physiological and experimental conditions available, it becomes possible to control and modify susceptibility relations to a considerable extent and so to determine more definitely than would otherwise be possible various factors concerned.

In the following sections the susceptibility relations observed under these various conditions are described and analyzed. The figures although diagrammatic are all drawn from animals actually observed and indicate the degree of contraction of the body-regions in each particular case. In each figure the intact portions are indicated by continuous outlines, the disintegrated portions by dotted shading, and arrows beside the figure indicate the direction in which disintegration is progressing. Since the course of disintegration in the tentacles is almost invariably basipetal and, with only occasional exceptions, very similar in all tentacles of an individual, the figures show the tentacles only so far as is necessary to indicate their condition in each case. Parts of body or stalk already disintegrated are usually drawn only so far as is necessary to indicate the course of disintegration in figures of later stages.

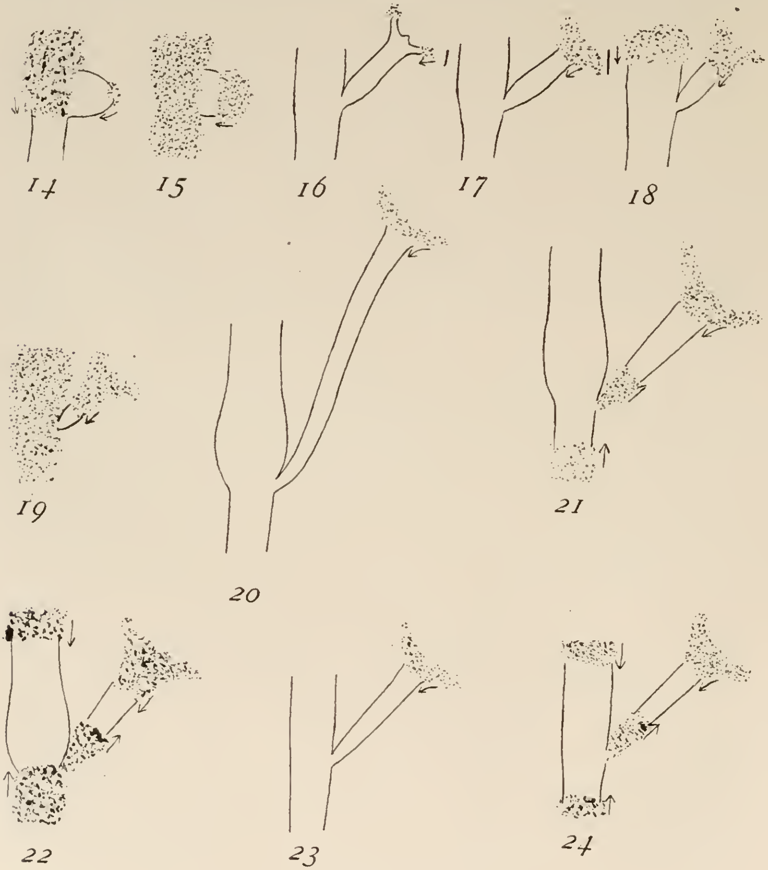
While the stages and progress of disintegration indicated in the figures concern primarily the ectoderm it should be noted that in well-fed animals the entoderm disintegrates at about the same time as the ectoderm, or in many cases somewhat earlier. Often disintegrated entoderm begins to flow out of the mouth before disintegration of the body ectoderm begins, and in the tentacles it can be directly observed that entodermal disintegration keeps pace with or follows very soon after disintegration of the ectoderm. In starved animals, however, the entoderm is distinctly less susceptible than the ectoderm, the difference between the two layers increasing up to a certain point with the progress of starvation. This difference between starved and fed animals is undoubtedly associated with the decrease in the metabolic activity of the entoderm in the absence of food. In the starving animal the functional activity of the entoderm must undergo very considerable decrease, while the functional activity of the ectoderm continues or may even increase with increasing "hunger."

The mesoglœa does not disintegrate, and the general form of the animal is retained even after disintegration, except where there is considerable movement of the fluid, but the replacement of cellular structure by cellular detritus, and the increase in transparency, except in the dyes, make it possible to distinguish without difficulty the occurrence and progress of disintegration.

The Primary Gradient in Buds.

The earlier stages of buds before motor activity has developed to any marked degree show the same susceptibility relations in all three species and in all directly lethal concentrations of all agents used. The earlier stages in which the bud is merely a slight protuberance of the body wall, usually show no gradient distinguishable from that of the region to which it is attached, but sometimes even these early stages show the basipetal gradient characteristic of later stages (Figs. 14, 15). As the development of the bud progresses, however, a distinct disintegration gradient, basipetal with respect to the bud axis, makes its appearance (Figs. 14, 15) and in early stages of tentacle-development, but before the stalk has developed, the gradient is uniformly basipetal in both tentacles and column (Figs. 16–19) and under the various experimental conditions, except in certain cases of strong contraction of the parent body or stalk to be mentioned below.

In still later stages, after the stalk has developed and the bud is nearly ready for separation, the course of disintegration varies according to conditions. Where the bud remains well extended and quiescent the gradient is basipetal as in the earlier stages (Figs. 16–19), but where the stalk undergoes marked contraction in the susceptibility agent the stalk region appears as a region of high susceptibility, disintegration beginning in it as soon or almost as soon as in the hypostome region. In such cases disinte-

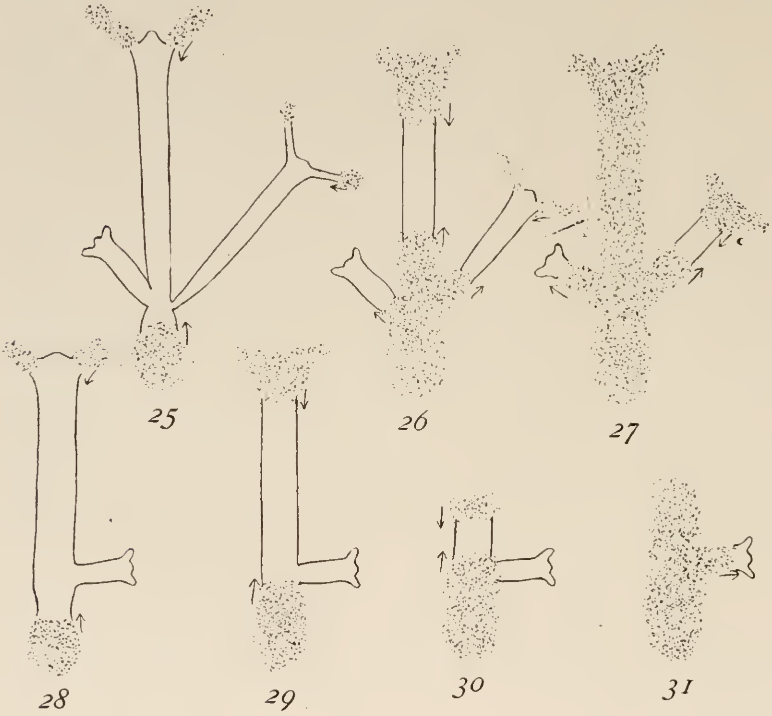


FIGS. 14–24.

gration of the stalk may begin at the base and progress acropetally, or may occur at once throughout most of the length of the stalk, but it is evident in all such cases that the stalk has become a region of relatively high susceptibility. In Figs. 20–22 a case of this sort in *H. vulgaris* is shown. In the earlier stages of disintegration (in KNC) the bud remained extended (Fig. 20) but later underwent considerable contraction, the stalk region contracting more than the body, and soon after the contraction the stalk began to disintegrate (Fig. 21), and in later stages disintegration progressed basipetally in the body and acropetally but more slowly from the stalk (Fig. 22), so that the two gradients met near the base of the body. Figures 23 and 24 show a similar case in *H. viridissima*. Here the contraction occurred shortly after the parent and bud were placed in the KNC, but, as in the preceding case, the stalk appears as a region of high susceptibility, and two gradients of disintegration progressing in opposite directions result. These cases are merely examples of what has been observed by us repeatedly in all three species.

In Figs. 25–27 an animal (*H. oligactis*) with two buds, one less the other more advanced, is shown. Here the more advanced bud, which has already developed independent motor activity, shows contraction of the stalk and double gradients, as in the cases of Figs. 20–22 and 23, 24. The earlier bud, however, which is much less motile and less independent of the parent body, shows an acropetal instead of the usual basipetal disintegration gradient. This is one of the exceptional cases referred to above. It will be noted that the stalk of the parent is strongly contracted and shows a high susceptibility. The reversal of the gradient in the earlier bud results from the spreading or irradiation into the bud of the excitation connected with the contraction of the stalk. The more advanced bud is more independent, *i. e.*, its own gradient is more fixedly established and it is less intimately connected with the parent, so that is it less affected by contraction of the parent stalk, although the contraction and early disintegration of its own stalk may perhaps be the result of irradiation of the excitation from the parent. Figs. 28–31 show a similar case of reversal of a bud gradient in *H. vulgaris*.

Reversals of this sort in buds have been repeatedly observed in *H. oligactis* and *H. vulgaris* and doutbless occur also in *H. viridissima*. Observations on the contraction of animals bearing buds in the earlier stages show very clearly that when marked contraction of the parent stalk or body or both occurs, some slight contraction of the bud may follow and in such cases progresses acropetally in the bud. In view of these facts, we believe that such reversals of bud gradients are to be interpreted as the expression of physiological relations which can be directly observed in the normal animals.



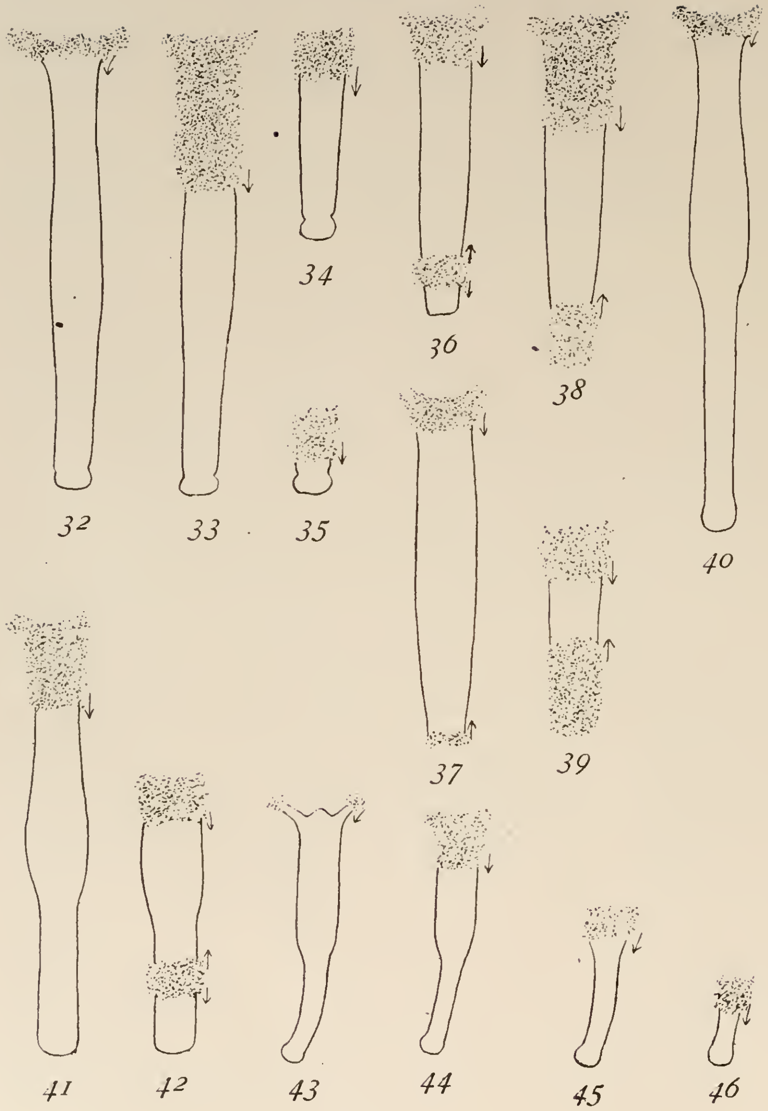
FIGS. 25–31.

The results in all agents show that the primary gradient in the bud is basipetal, but that, even in the earlier stages, it may be modified or reversed by contractile activity in adjoining regions of the parent animal and in later stages by its own contractile activity. In the higher concentrations of anesthetics and in the irritant dyes certain other modifications of the susceptibility relations in the later bud stages occur, but since they are similar to the modifications in full grown animals under the same conditions, their consideration is postponed to a later section.

THE GRADIENTS IN ATTACHED QUIESCENT ANIMALS.

For present purposes "attached animals" are animals which have been left undisturbed in Syracuse dishes for several hours, usually over night, and are firmly attached to the glass of the dish. Animals attached to the surface film or to air bubbles are not included. After the preliminary period of attachment, most of the water is carefully drawn off, the agent added with a pipette and the dish covered without air bubbles, great care being taken to disturb the animals as little as possible. Since cyanide is the only agent among those used in this study which does not produce some considerable degree of excitation, it is employed in various concentrations from *m*/1,000 to *m*/200 with the most satisfactory results, where it is desired that the animals shall remain quiescent. The change of fluids is often accomplished without inducing any strong contraction, and if the animals contract at all, they usually extend again at once or remain at least moderately extended until death, unless otherwise disturbed.

Under these conditions individuals of *H. viridissima* and *H. vulgaris* show the primary gradient, disintegration progressing basipetally throughout tentacles, body and stalk (Figs. 32–35). In cases where the experiment is not entirely successful and any considerable degree of contraction of the stalk occurs, disintegration progresses basipetally as before in tentacles and body, but as disintegration of the hypostome begins, or somewhat later, disintegration also begins in the stalk somewhere above the foot and progresses in both directions (Fig. 36), or in some cases at the foot itself and progresses acropetally (Figs. 37, 38), death occurring last in or just apical to the basal region of the body (Fig. 39). Figs. 40–42 show a particularly clear case of this sort in *H. vulgaris*. Here the attached animal remained extended during the disintegration of tentacles and hypostome and the usual basipetal gradient appeared (Fig. 40), but at a later stage the stalk underwent considerable contraction (Fig. 41), probably in consequence of movements of the dish to and from the microscope

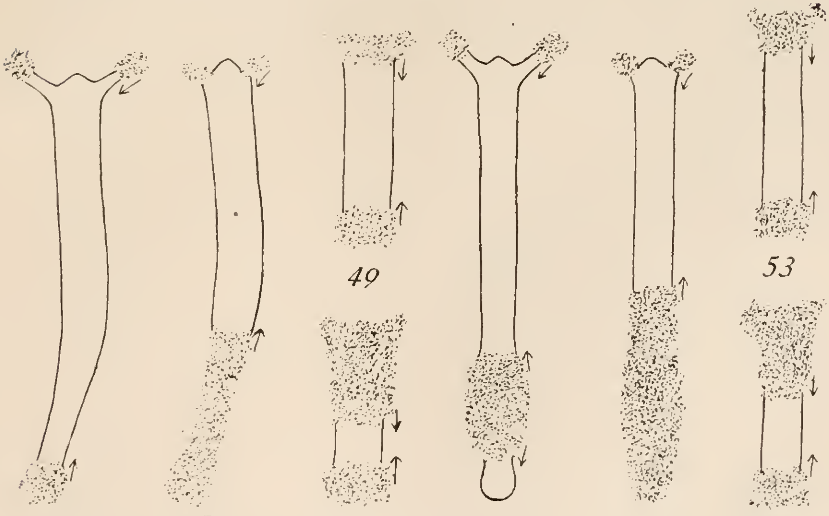


Figs. 32–46.

stage, and soon after this contraction disintegration began in the stalk above the foot (Fig. 42) and progressed in both directions from this level with the usual final result.

H. oligactis differs markedly from the other two species in that attached quiescent animals in all cases observed by us, with a single exception, show a secondary gradient or gradients in the stalk, and this secondary gradient extends further acropetally than in the other two species. In the first place, it is much more difficult to obtain attached quiescent individuals of *H. oligactis* for observation, for no matter how firmly attached and inactive they may be preceding the addition of the agent, most of them detach themselves either when the water is drawn off or when the agent is added. In many cases attachment occurs again in the solution within a few moments but is it preceded by more or less motor activity of the stalk. Moreover, in the individuals which remain attached the stalk apparently always undergoes some contraction.

In fact, probably the only completely successful attempt to obtain an attached quiescent individual for susceptibility determination is the case of the single exception mentioned above. This was a small animal, only recently separated from the parent body, which remained attached and quiescent during the whole period of observation and showed only the primary basipetal gradient (Figs. 43–46) like attached quiescent individuals of *H. viridissima* and *H. vulgaris*. Since this animal was not full grown and not far beyond the bud stage, and since the later bud stages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Figs. 47–54.

often show a purely basipetal gradient, even after the stalk is distinguishable morphologically (p. 194), it is at least possible, that in this case the stalk had not attained complete specialization as a contractile region.

In all other cases of full grown attached individuals of *H. oligactis* more or less contraction occurred in the stalk, and the secondary gradient oi gradients appeared according as the secondary disintegration began at the foot (Figs. 47–50) or above it (Figs. 51–54) and the acropetal gradient in all cases extended at least to the middle of the body (Figs. 50, 54) and often farther apically.

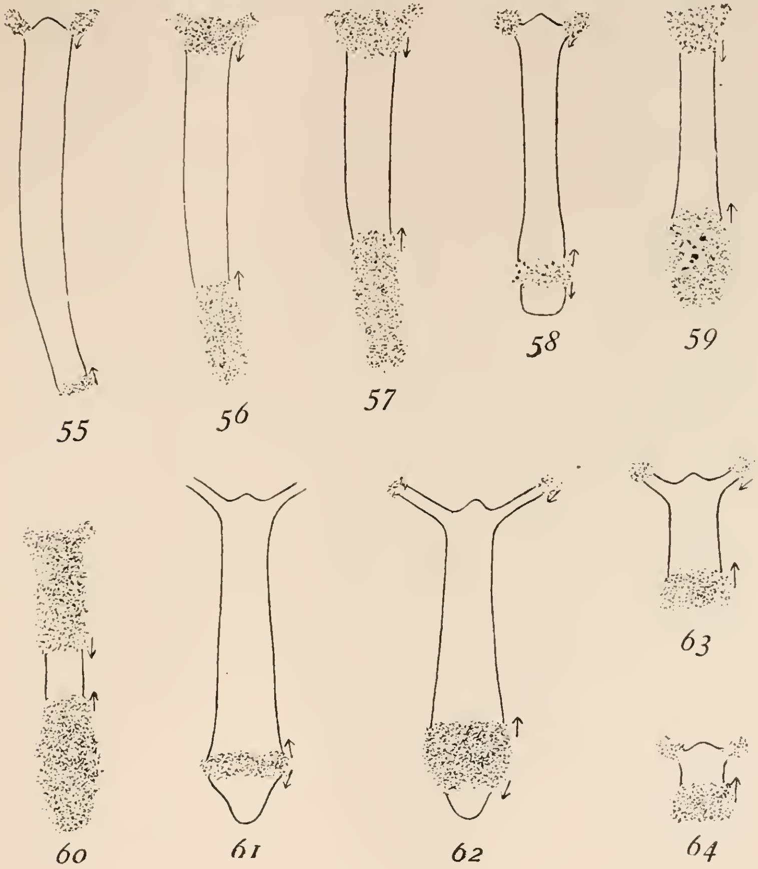
While these observations on the susceptibility of attached, quiescent animals indicate that the stalk of *H. oligactis* is more highly specialized than that of the other species, at least as regards its sensitiveness, they do not prove that it is permanently a region of high susceptibility.

The Gradients in Detached and Recently Attached Animals Without Special Excitation.

In all three species the activity of the stalk is usually greater in the detached than in the attached animals even without any special external excitation, and the early appearance of disintegration at the foot (Figs. 55–57, *H. oligactis*) or in the stalk above it (Figs. 58–60, *H. vulgaris*) is the characteristic result as in attached animals with stalk contraction (Figs. 36–42 and 47–54). Here again KNC is the most satisfactory agent among those used, although the lower concentrations of anesthetics and dyes give the same results. In general the more active or more strongly contracted the stalk, the earlier it begins to disintegrate. In *H. oligactis* cases like 61–64 are not infrequently seen. Here the stalk is strongly contracted and the body progressively less contracted from the base apically, and disintegration begins in the stalk even earlier than the tentacles and progresses acropetally to the hypostome, *i. e.*, complete reversal of the gradient occurs, except in the tentacles. Such reversal has not been observed in the other species.

Rarely, however, detached individuals of *H. viridissima* and *H. vulgaris* remain quiescent with extended stalk and show the primary basipetal gradient like attached quiescent animals (Figs. 32–35). Cases of this sort have not been observed in *H. oligactis*.

Animals which have been attached only a short time, *e. g.*, an hour or two, preceding exposure to the reagent, and animals which attach themselves in the solution, usually show gradients like those of the detached animals, *i. e.*, basipetal in tentacles and body and acropetal from the foot, or in both directions from some level of the stalk. In these cases there is usually more or less activity of the stalk for a time after attachment, and apparently the condition of excitation persists for some time after



Figs. 55–64.

muscular activity, though how long has not been determined. Sometimes, however, newly attached individuals of *H. viridissima* and *H. vulgaris* remain quiescent and well extended and show the primary basipetal gradient throughout.

SUSCEPTIBILITY IN RELATION TO GENERAL EXCITATION, ANESTHESIA AND MUSCULAR PARALYSIS.

It was noted above (p. 191) that alcohol, ether and the dyes used produce primarily more or less excitation, as indicated by increased muscular activity. This condition is of course followed sooner or later by depression and muscular paralysis, and, according to the agent and concentration used, death and disintegration may occur under conditions ranging from extreme muscular relaxation to extreme contraction and rigidity. The susceptibility relations under these conditions afford further evidence that muscular activity is an important factor in the susceptibility of at least the ectoderm of hydra.

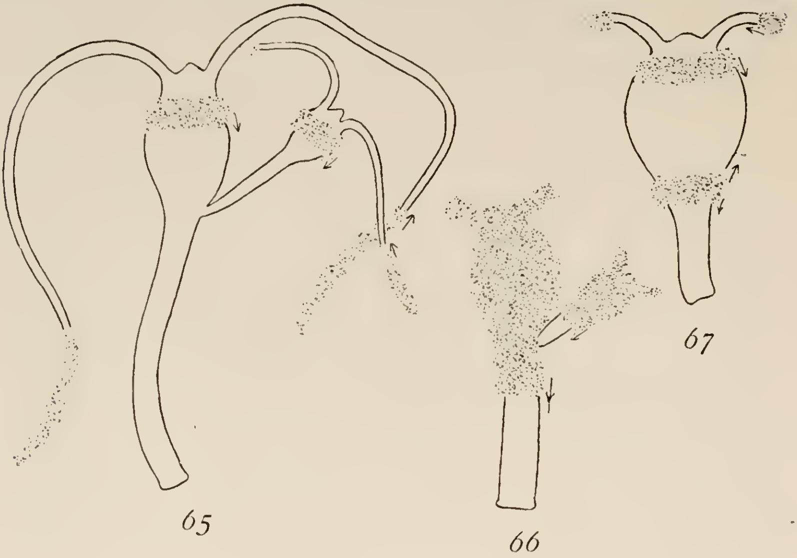
The degree of general excitation and the rapidity with which depression and paralysis follow excitation and therefore the regional differences in susceptibility depend to a considerable extent upon the concentration of the agents used. The lowest killing concentrations of alcohol and ether produce but little excitation, and in these disintegration and death usually follow the same course as in detached animals in cyanide (p. 200). Neutral red also, in the concentrations used, produces comparatively little excitation, and the susceptibility relations in this agent usually show no special modifications. Methylene blue produces a considerably greater degree of excitation and Janus green is an extreme irritant, and since these dyes accumulate in the cells, even when the external concentration is very low, a considerable degree of excitation occurs sooner or later, even in very low concentrations, but the higher concentrations bring about excitation more rapidly and to a more extreme degree than the lower. Only in extreme dilutions of these dyes is the course of disintegration like that in detached animals in cyanide.

In all these agents, the earlier bud stages preceding the development of independent motor activity show, as already noted, the same basipetal gradient as in cyanide, except where the bud gradient is partially or wholly reversed by the irradiation of excitation from the parent animal into the bud (p. 195). This primary gradient is then the same in all agents used. The late bud stages show essentially the same relations as the full grown animals and are considered with the latter.

Since the modifications of the primary gradient under consideration appear most distinctly and under the widest range of conditions in *H. oligactis*, they are first described as they occur in this species, and this description serves as a basis for comparison with the other species.

Experimental conditions and results with alcohol and ether are briefly as follows: the higher concentrations, ether 2–3 per cent., alcohol 3–5 per cent., are added rapidly to well extended animals in a little water and the dishes covered with exclusion of air. The tentacles and hypostome region are usually completely paralyzed in the extended condition before they are able to contract at all, the body begins to contract and usually succeeds in completing a strong contraction, but after that shows no further movement, and the stalk, which under normal conditions usually contracts later than the body, is either paralyzed before it can contract, or transmission of the stimulus from the body and excitation of the stalk are inhibited by the anesthetic before the stalk reacts. Fig. 65 shows an animal with advanced bud in the condition characteristic of this method of procedure. The tentacles are completely paralyzed, the body has contracted strongly and the stalk remains extended in both parent and bud. Under these conditions the disintegration of tentacles and hypostome is delayed as compared with that of the body, disintegration beginning in a zone just below the tentacle bases and in the tips of the tentacles at about the same time (Fig. 65). Disintegration progresses basipetally in both tentacles and body and also through the stalk (Fig. 66), the foot being the last region to disintegrate. The stalk usually undergoes a very slow decrease in length shortly before, or when disintegration begins (cf. Figs. 65 and 66). This is perhaps a response of the muscular portions of the cells to direct excitation by the reagent, or it may be an expression of the elasticity of the mesoglœa or other tissues, appearing after complete muscular paralysis.

In some cases in these concentrations of ether and alcohol and more frequently in somewhat lower concentrations, the stalk may undergo partial contraction at its apical end before paralysis occurs and in such cases the apical region of the stalk appears as a second region of high susceptibility (Fig. 67). In general, the lower the concentration of alcohol and ether used, the less rapid and less complete the paralysis of tentacles and hypostome, the greater the contractile activity of the stalk, and the closer the behavior both in motor activity and in the course of disintegration to the detached animals in cyanide.



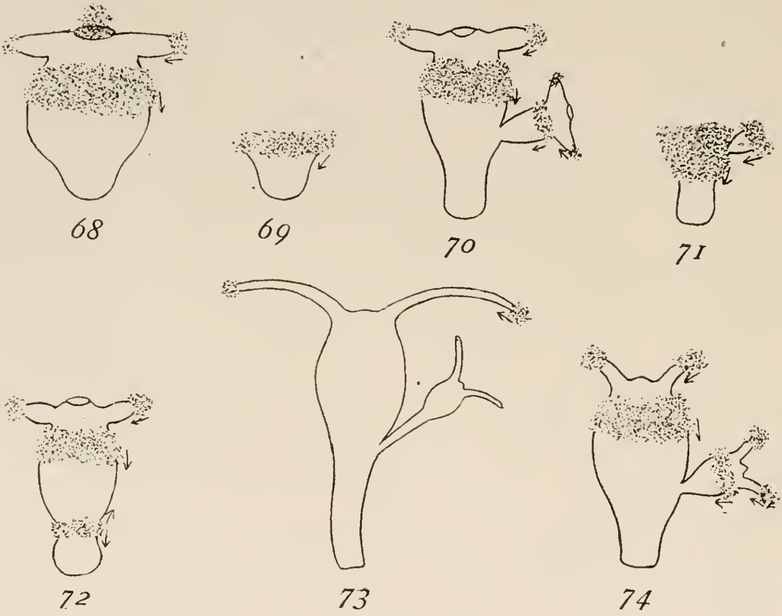
Figs. 65–67.

These experiments with alcohol and ether indicate first that inhibition of excitation and the resulting paralysis occur earliest in the tentacles and hypostome region. In concentrations where paralysis does not occur at once it can be seen that the paralysis progresses basipetally in the tentacles. In the column also the paralysis apparently progresses basipetally, although the fact that the body usually contracts before the stalk in the primary excitation may mask this relation in the higher concentrations. Apparently then the susceptibility as regards the paralyzing action of these anesthetic agents shows a basipetal gradient like the primary gradient.

The second conclusion from these experiments is that disintegration and death are retarded in those regions which are paralyzed immediately or very early, as compared with those regions which show marked muscular activity before paralysis, so that disintegration may begin in the body below the tentacles (Fig. 65), and often also in the stalk, while the tentacles are mostly and the hypostome wholly intact.

The course of disintegration in methylene blue and Janus green is essentially the same as in alcohol and ether. In these dyes, however, the excitation is more marked and in the lower concentrations (methylene blue, 1/10,000 or less, Janus green 1/50,000 or less) may continue for hours, but is finally followed by paralysis and rigidity in a state of more or less extreme contraction. This paralysis and rigidity, which perhaps resembles muscle rigor, appears first at the tips of the tentacles and progresses basipetally in tentacles, body and stalk. The motor behavior in these dyes consists at first merely of elongation, contraction and other movements of all parts, but as staining progresses, the behavior comes to resemble more and more closely the disgorging reaction of normal animals. The mouth becomes permanently open, the hypostome flattened, the tentacles stand at right angles to the body, and the body itself undergoes repeated contraction, while the stalk is much less active and only very gradually attains a strongly contracted state. It may be noted that these dyes accumulate in the entoderm as rapidly as or even more rapidly than in the ectoderm, and the entoderm often begins to disintegrate before the ectoderm. To all appearances they irritate the entoderm and so give rise to the disgorging reaction. But whatever the exact physiological condition may be, these dyes produce relatively early paralysis of tentacles and hypostome, extreme activity of the body and less activity of the stalk before the final rigidity death and disintegration occur. As regards the course of disintegration, the result is much like that in alcohol and ether, disintegration beginning in the body below the tentacles and progressing basipetally while the tentacles are mostly and the hypostome wholly intact (Figs.

68, 69, Janus green 1/10,000). In Fig. 68 disintegrated entoderm is issuing from the mouth. Figs. 70 and 71 show another case, an animal with advanced bud in Janus green 1/200,000 approximately. In this low concentration the contraction is less extreme but the course of disintegration is the same as in the higher concentration. In these very low concentrations where the body contractions are less extreme, disintegration often appears at the anterior end of the stalk at about the same time as in the body (Fig. 72). In methylene blue the picture as regards both



Figs. 68–74.

behavior and disintegration is essentially the same, but the degree of irritation with a given concentration is less than in Janus green. In these dyes, as in alcohol and ether, the relatively early paralysis of tentacles and hypostome, the great activity of the body and the less extreme activity of the stalk alter the regional susceptibility relations so that disintegration and death begin relatively early in the body and often in the stalk, as compared with tentacles and hypostome.

As regards *Hydra viridissima* the results in ether 2–3 per cent.

(Figs. 73–74) and in Janus green are essentially the same as in *H. oligactis.* The susceptibility of *H. viridissima* to alcohol, however, is very low, muscular activity continuing for 24 hours or more in 3 per cent., for 3–4 hours in 5 per cent. and for a minute or two even in 10 per cent. Alcohol produced but little excitation in this species and the disintegration gradient in alcohol is either basipetal throughout or double, as in detached animals in cyanide (pp. 200–202). The reason for the very low susceptibility to alcohol of this species we have not been able to determine. To cyanide and to ether it is somewhat more susceptible than *H. oligactis.* In methylene blue *H. viridissima* shows less excitation than *H. oligactis* and the course of disintegration is like that of detached animals in non-excitant agents. In Janus green, however, marked excitation occurs followed by paralysis beginning in the tentacles and hypostome and the course of disintegration is like that in *H. oligactis.* In other words *H. viridissima* shows this modification of the primary gradient only in those agents of this group which act most rapidly and most powerfully on *H. oligactis.*

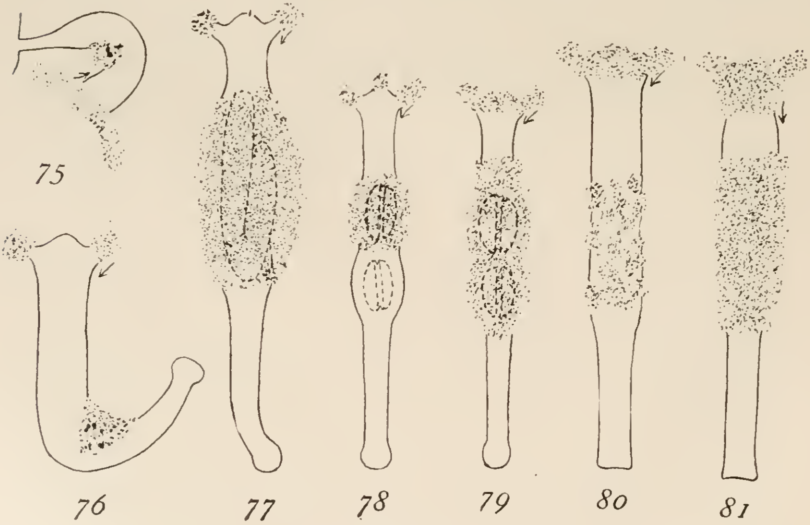
H. vulgaris is apparently still less sensitive to the particular effects of this group of agents, and its motor behavior is less altered by them and only in Janus green, the most irritating of all, has the special modification of the gradient been observed. In this agent there is marked excitation ending in strong contraction and rigidity, and the course of disintegration is like that in *H. oligactis* (Figs. 68–72).

The fact that alcohol, ether, methylene blue and Janus green all produce the same modifications in the course of disintegration and death in *H. oligactis* suggests the possibility of a certain similarity in their action. All produce more or less excitation at first and later paralysis, though the period and degree of excitation differ widely. While the data are not adequate to permit definite conclusions, it seems evident from the observed facts that these agents act primarily upon the receptive or sensory and perhaps the transmissive mechanism of hydra and that the modifications of motor behavior and the course of disintegration are the indirect results of this action. We should expect the action of alcohol and ether to be of this character, methylene blue is a more or less specific agent for nervous tissue in higher animals and the extreme irritation produced by Janus green indicates that it also affects this mechanism in some way. If this suggestion is correct, the basipetal course, first of excitation and second of paralysis, may indicate regional gradation in susceptibility in the receptive or nervous apparatus of hydra. Moreover, on this basis the differences between the three species suggest that this apparatus shows a higher degree of regional specialization in *H. oligactis* than in the other two species, and that *H. viridissima* is at least somewhat more sensitive or excitable than *H. vulgaris*.

Susceptibility in Relation to Unsymmetrical Contraction.

Usually all the tentacles of an individual behave in much the same manner in a given agent, but sometimes one or more tentacles contract more strongly than the rest. In such cases the more strongly contracted tentacle is usually more susceptible and disintegrates earlier than those which do not contract.

In cases also where the body becomes strongly bent and retains that position, disintegration occurs earlier on the more contracted than on the less contracted side. Fig. 75 shows a case of this



Figs. 75–81.

sort in a late bud and Fig. 76 another in a full grown animal. Numerous other similar cases have been observed. It is of course possible that both the unsymmetrical contraction and the early disintegration indicate a local injury, although none was observed in these and other similar cases. Visible injuries to the body wall do become centers of early disintegration, undoubtedly in consequence of the excitation produced by the injury, and they may also bring about local contraction, though in many cases they do not. While local injury cannot therefore be entirely excluded as a factor in such centers of early disintegration, it seems evident that the early disintegration is the result of local excitation, whether this be due to injury or to muscular contraction or both.

LOCAL EFFECTS OF DIGESTIVE ACTIVITY ON SUSCEPTIBILITY.

When recently taken food is present in a particular region of the digestive tract a region of early disintegration appears corresponding at first to the region occupied by the food. Fig. 77 shows the case of a large individual of *H. oligactis* containing a recently ingested Chironomid larva as indicated. The region of the body in which the larva lies shows a very high susceptibility, both ectoderm and entoderm disintegrating at the same time as the tentacles and much earlier than other body regions.

Figs. 78 and 79 show another somewhat similar case. This animal, when isolated from the stock, had in its digestive tract the shell of an entomostracan, the soft parts of which were wholly or largely digested. An hour before the susceptibility determination entomostraca were placed in the dish with the animal and one was ingested. In the figure the shell of the first entomostracan is below the second newly ingested one. The body region containing the second animal which is in process of digestion shows a high susceptibility, both ectoderm and entoderm disintegrating at the same time as the tentacles (Fig. 78), while the lower region containing the animal which has already been largely or wholly digested is less susceptible, but still more susceptible than other regions of the body and stalk (Fig. 79). After the stage shown in Fig. 79 the disintegration did not extend from the regions concerned in digestion, but progressed basipetally from the hypostome and also began at the foot and progressed acropetally, *i. e.*, the susceptibility of these regions was like that of many other detached animals.

Figs. 80 and 81 show the course of disintegration in an animal which contained a recently ingested chironomid larva when it was placed in cyanide, but disgorged the larva within the first five minutes. Although disintegration did not begin until an hour later, the region of the body in which the larva had been began to disintegrate as soon as the tentacles (Fig. 80) and was completely disintegrated before any other part of the stalk or body except the most apical region (Fig. 81).

These and numerous other similar cases observed indicate that the activity of the entoderm in digestion extends to the ectoderm of the region concerned. The relations already described between susceptibility and muscular contraction indicate that more or less transmission or irradiation of local excitations inducing contraction occurs, and these cases of high susceptibility associated with digestive activity indicate an irradiation to the ectoderm of the entodermal excitation produced by food. Probably no excitation is sharply localized in hydra.

The Color Gradients in Potassium Permanganate.

It has been found by one of us that the axial gradients in physiological condition in various plants and animals can be demonstrated as color gradients through the reduction of potassium permanganate to MnO_2 by the living protoplasm, the regions of high susceptibility and high oxidation rate reducing the permanganate more rapidly and so being stained more rapidly or more deeply by the MnO_2 (Child, '19). Some observations with permanganate have been made on all three species of hydra but the agent produces a great irritation even in very low concentrations (*m*/50,000) and the resulting contraction is so extreme that it is difficult to distinguish anything more than the general color gradations in full grown animals and later bud stages.

The earlier bud stages which possess little or no contractility show a distinct basipetal color gradient in permanganate, corresponding to the primary susceptibility gradient. In the tentacles also the color gradient is basipetal like the susceptibility gradient. Fully developed animals almost invariably detach themselves at once in permanganate and always undergo extreme contraction. They show double color gradients, basipetal from the apical end and acropetal from the foot, but modifications of these gradients by contractile activity of stalk or body cannot be observed because extreme contraction occurs in all cases. As far as they go, however, these observations on color gradients agree with and confirm the data on susceptibility.

SUSCEPTIBILITY IN RELATION TO PHYSIOLOGICAL AGE.

The view has been advanced by one of us that a process of dedifferentiation and rejuvenescence is associated with agamic reproduction, and that the individuals arising from a part of a preexisting individual by process of budding, fission, etc., are physiologically younger than the individual from which they arose (Child, '15*b*, especially Chaps. VI., X.). In general the rate of the fundamental metabolic processes decreases with advancing senescence, and the agamically produced individuals show in their higher rate of growth, their higher direct susceptibility and greater capacity for acclimation, and in all cases thus far examined their higher rate of CO_2 production, that they possess a higher metabolic rate than the individuals from which they arose, and their morphological and cytological condition as compared with that of the parent individual also indicates that rejuvenescence has occurred.

If this view is correct, and if susceptibility to toxic agents is a criterion of physiological or metabolic condition, we might expect to find that the earlier bud stages of hydra are more susceptible than the animals from which they arise and that susceptibility decreases with advancing development.

As a matter of fact, however, the early bud is almost invariably less susceptible than the parent animal and the susceptibility of later bud stages is greater than that of earlier stages and is often equal to that of the parent. On the other hand, the "young" separate animal recently developed from a bud shows in general a higher susceptibility than the "old" full grown animal. As regards the early bud, Fig. 14 shows the usual condition, in which disintegration of the parent body occurs before that of the bud. In Figs. 18 and 19, a somewhat later bud stage, the disintegration of the body is completed before that of the bud. In Figs. 20 to 22 the susceptibility of a late bud is about equal to that of the parent and in Figs. 23 and 24 the same is true. In Figs. 25–27 both the earlier and later bud are less susceptible than the parent, but here the susceptibility of the earlier bud is increased by irradiation of the excitation from the parent body. Fig. 28 shows another case where susceptibility of a rather early bud stage is less, even with gradient reversed by irradiation of excitation, than that of the parent. In Figs. 65 and 66, 70 and 71, 73 and 74 the susceptibility of advanced buds is seen to be about equal to that of the parent.

This apparent discrepancy in the results of the susceptibility method finds a very simple explanation in the total or almost total absence of motility in the earlier bud stages and its gradual development. In view of all the evidence presented above to show the influence of muscular activity upon susceptibility, it cannot be doubted that the development of motility in the bud compensates the effect of progressive development as regards susceptibility. The non-motile cells of the early bud, although physiologically younger than the cells of the late bud or the separate animal, are less susceptible than the cells which have developed a functional contractile mechanism. The susceptibility method does not of course distinguish between the intrinsic high metabolic rate of embryonic cells and the "functional" high rate of differentiated cells resulting from excitation. In these animals, where the muscles are merely portions of the cells and where excitation and muscular activity evidently alter the condition of the cells so greatly, the functional differences in metabolic rate usually overbalance the intrinsic differences associated with differences in physiological age.

When the bud has become a separate animal with fully developed excitability and motility, it is fairly comparable with the full-grown animal and at this time susceptibility is greater than that of the latter, *i. e.*, the functional characteristics being developed in both, the age differences now appear in the greater susceptibility of the younger animal.

This interpretation of the susceptibility relations of buds and parents and earlier and later stages of development is based on wide observation by both of us, and agrees well with the other data on susceptibility. In a later paper it will be shown that similar relations exist in other hydrozoa.

The Susceptibility of Sexual Animals.

A few observations have been made on gonad-bearing animals with ether as agent. In all cases observed, the gonads are less susceptible than other regions of the animal. As in the case of early buds, the low susceptibility of the gonads is perhaps not a correct criterion of their physiological condition as compared with other parts of the body, for the reproductive cells are of course entirely separate from the muscular mechanism and are probably not very greatly affected by excitation and contraction in other cells. The number of sexual individuals available has been too small to permit definite conclusions concerning the comparative susceptibility of sexual and non-sexual animals, though the few data at hand indicate that sexual animals are in general less susceptible than non-sexual.

Susceptibility in Relation to Concentration.

Since the present paper is primarily concerned with other problems than those connected with concentration of agents, the data on concentration are incidental and presented merely to show approximately how rapidly the processes of disintegration occur in concentrations most commonly used. There is of course some individual variation in susceptibility, doubtless dependent on uncontrolled factors such as nutritive condition, physiological age, motor activity, etc., and the course of disintegration differs under different conditions, as already described. A few characteristic data for large, well fed, asexual individuals of *H. oligactis* at temperature 20°–22° C. are tabulated for illustration and comparison.

The concentrations given in the table for KNC, alcohol, and ether are of course only approximate. All solutions of these agents were freshly made in every case and loss from volatilization was prevented as far as possible. As regards methylene blue and Janus green, the tabulated data are all from solutions of the same sample of each. Different preparations of these

TABLE.

Agent.	Concentration.	Time in Hours from Beginning of Experiment.	
		Beginning of Disintegration.	Completion of Disintegration.
KNC	*m*/1,000	2– 3	10–18
	m/400	1 ±	6– 9
	m/200	½ ±	4– 6
	m/20	1– 5 minutes.	¼–½
Alcohol	2 per cent.	Usually not directly lethal within 24 hrs. except for tips of tentacles.	
	3 per cent.	5– 8	18–24
	4 per cent.	3 ±	8–10
	5 per cent.	1 ±	4– 6
Ether	1 per cent.	½– 1	3– 5
	2 per cent.	¼ ±	½ ±
	3 per cent.	5–10 minutes.	¼–½
	0.0005 per cent.	12–16	22–24
	0.01 per cent.	8–12	18 ±
	0.02 per cent.	6– 8	10–18
	0.0002 per cent.	2 ±	18–20
	0.002 per cent.	1 ±	4 ±
	0.01 per cent.	½ ±	2 ±
	0.05 per cent.	at once	¼

dyes showed some difference in degree of toxicity. The plus or minus sign following the figures denoting times indicates that the exact time was not observed. The data for neutral red are not tabulated because the gradual precipitation of the dye in aqueous solution makes it impossible to maintain a constant concentration, but in a concentration originally approximately 0.005 per cent. of the sample used disintegration began after 1–2 hours and was completed after 6–7 hours.

Young, recently separated animals disintegrate in half to two thirds the times given in the table, animals regenerated from pieces resemble the young animals in susceptibility, and in starved animals the ectoderm is more susceptible than in well-fed animals while the entoderm, being in large measure functionally inactive, is usually less susceptible than in well fed animals.

In general the susceptibility of *H. viridissima* and *H. vulgaris* to a given concentration of a particular agent is somewhat greater than that of *H. oligactis*, but the low susceptibility of *H. viridissima* to alcohol (p. 207) is apparently an exception to this rule.

Comparisons between the species are best of limited value because various physiological conditions play so large a part in determining the susceptibility of the individual.

DISCUSSION.

The data show first, that a primary axial gradient in susceptibility exists in the three species of hydra, with the region of highest susceptibility at the apical end, and second, that this gradient is variously modified by the functional activity and particularly by muscular contraction in the various regions. If the view advanced in various papers (see references, Child, Hyman) and supported by various lines of evidence, that susceptibility is in a general way a criterion of metabolic and physiological condition, is correct, the facts indicate that there is in hydra, as in other axiate organisms examined, an apico-basal metabolic gradient, with region of highest metabolism at the apical end. It is perhaps scarcely necessary to repeat once more that the differences in metabolic rate are only one feature of such a gradient. Associated with them are of course differences in protoplasmic condition of various sorts. The important point is that the apico-basal axis in hydra, like the axis of other organisms, appears as a gradient in relation to the action of external agents. Discussion as to what constitutes the primary feature of such a gradient is at present of little value, since metabolism and protoplasm are always associated in active living organisms. Likewise, the question how each particular agent acts on protoplasm is of minor importance for the general conception.

As in other axiate forms, the primary gradient corresponds to a relation of physiological dominance and subordination, the region of highest susceptibility being to a greater or less degree a region of initiation and control. In hydra this is true, both of the tentacle and of the column. The stalk represents physiologically a more or less specialized contractile region secondarily developed from the basal portion of the body, but the primary gradient is altered by the contractile activity of the stalk, not by its mere presence, except possibly in *H. oligactis*, though even here the high susceptibility of the stalk observed in most cases is apparently associated with more or less muscular activity.

Whatever the terms in which we may finally define the axial gradient, its existence and significance cannot be doubted. In hydra, as in other forms examined (see references, Child, Hyman), it is the earliest indication of the body axes, it is certainly associated with quantitative differences in metabolic and general physiological condition along its course, and physiological dominance and subordination, as well as local specialization and differentiation exist or arise in definite relation to it. In the light of all the known facts, the only possible conclusion is that the gradient is the simplest physiological expression of the axial relation in organisms. In other words, this axial relation in its simplest terms is a quantitative gradation in the condition of living active protoplasm.

The modifications of the gradient in hydra by contractile and digestive activity are significant in that they constitute further evidence for the value of the susceptibility method as a means of investigating metabolic condition or "physiological state" in simple organisms. Since the contractile mechanism in hydra is merely a part of the body cell, excitation and muscular contraction affect the whole cell to some extent, and the altered condition of the cell appears in its altered susceptibility. Similarly the spreading or irradiation of excitation with both local muscular contraction and local digestive activity also appears in the susceptibility relations of different regions. The simplicity and diffuse character of the organization of hydra make it of particular interest in this respect, because each excitation produces a more or less diffuse effect.

The experimental data indicate further that a greater degree of excitation and change in physiological state is associated with the muscular activity which brings about contraction of tentacles, body or stalk, than with that concerned in their extension. Extension is doubtless due, at least in large part, to the activity of transverse or circular muscle fibers, but the process is much less rapid and less vigorous than that of contraction and occurs in the absence of strong external excitation, while contraction is induced by external excitation. Apparently the longitudinal muscles are more numerous, or more powerful, or their contractile activity is associated with more intense excitation, and it is this activity, or conditions associated with it, which play the chief part in modifying the primary susceptibility relations.

On the basis of studies of oxygen consumption and susceptibility in protozoa, E. J. Lund ('18*a*, *b*, *c*) has criticized the conclusion that susceptibility to cyanide is a measure of rate of oxidation or of metabolism. He has found, first, that *Paramecium* in cyanide shows no decrease in oxygen consumption until cytolysis or disintegration occurs, and second, that in starving *Paramecia* oxygen consumption decreases, while susceptibility increases.

In the absence of any attempt to account for the fact that his results apparently disagree completely with current theories of cyanide action, and of convincing evidence that his experiments are controlled with sufficient care, his negative results require further confirmation. But, even assuming that his experimental data are correct as they stand, he has apparently failed to recognize an essential point, viz., that susceptibility as measured by the progress of death and disintegration concerns primarily the ectoplasm (Child, '14*b*) or the body-surface and body-wall (Child, '15*b*, p. 75). Statements to this effect have been made repeatedly in the work on susceptibility.[1] It is highly probable that in *Paramecium* the oxidation in the thin layer of ectoplasm is only a very small fraction of the total oxidation, and that, therefore, the decrease in oxygen consumption resulting from the action of cyanide on the ectoplasm is not sufficient to appear with certainty in the total oxygen consumption, with the small amount of material used in his experiments. As soon as the ectoplasm begins to disintegrate and the entoplasm is directly exposed to the action of the cyanide, Lund finds a very marked decrease in oxygen consumption, *i. e.*, KNC does decrease oxidation at least in the entoplasm in his experiments. The susceptibility method, on the other hand, shows only the conditions in the ectoplasm. In short, Lund's data concerning the effect of cyanide on oxygen consumption in *Paramecium* are negative and inconclusive, except as regards the entoplasm, where they are positive and in agreement with current theory. They do not, therefore, justify his conclusion concerning either the action of cyanides on oxidation in *Paramecium*, or the results of the susceptibility method. In papers soon to be published we shall show that KNC produces a very marked decrease in both oxygen consumption and carbon dioxide production in *Planaria*.

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[1] Lund's attention was called to this point in personal correspondence soon after the preliminary report of his work appeared (Lund '18*a*).

His conclusions concerning oxygen consumption and susceptibility during starvation indicate that he has failed to take into account a second important fact, viz., that metabolic conditions in ectoplasm and entoplasm may be different and may change more or less independently. As a matter of fact Lund's data on starvation metabolism in *Paramecium*, as far as they go, are in full agreement with, though much less complete than, the results of our own studies made during the last two years on starvation metabolism in *Planaria*, but not yet published. While extended discussion and criticism along this line are postponed until publication of our own results, it may be noted here that the apparent discrepancy between susceptibility and metabolism appears in *Planaria* as well as in *Paramecium*, but as regards *Planaria*, it has been possible to demonstrate that it is only apparent. In well fed animals the metabolism in the alimentary tract is a very large part of the total, and during the early stages of starvation this metabolism at first decreases very rapidly as the alimentary tract becomes less and less active functionally. During this period, however, the functional activity and metabolism of the surface and body wall continue and the susceptibility method shows that the susceptibility of the body wall remains unchanged or increases slightly, but it also shows that the susceptibility of the alimentary tract as compared with that of the body wall decreases to a very marked degree. In other words, if we take into account the susceptibility of the alimentary tract as well as that of the body wall, which the data published in earlier papers did not do (Child, '14*a*, '15*b*, Chap. VII.), the discrepancy between the results on susceptibility and those on metabolism disappears.

In the more advanced stages of starvation, concerning which Lund presents no data, perhaps because *Paramecium*, being a single cell, dies before it reaches such stages, when all food and all reserves are gone, and the animal is living upon its own protoplasmic substance, the total metabolism begins to increase, and sooner or later attains a higher level than in the original animal. During this period susceptibility of both body wall and alimentary tract increases, but the susceptibility of the latter always remains lower as compared with that of the body wall in the starved than in the fed animal.

The apparent discrepancy between the susceptibility and metabolic methods results simply from the fact that the alimentary tract, and doubtless the entoplasm in *Paramecium* soon becomes practically inactive functionally in starvation while the body wall does not. Consequently total metabolism may decrease while susceptibility of the body wall increases or remains unchanged, but later both the susceptibility of body-wall and alimentary tract and the total metabolism increase.[1]

This discussion is sufficient to make it at least highly probable that Lund's conclusions result rather from inadequate analysis of his own experimental data in the light of the known facts, than from any real discrepancy between the results obtained by the susceptibility method and by direct measure of oxidation. He has failed to take account of two very essential facts: first, that ectoplasmic or body-wall oxidation is very far from being total oxidation and second that the rate of oxidation in ectoplasm and entoplasm or in body-wall and alimentary tract may vary independently, that of the alimentary tract or the entoplasm undergoing marked decrease in starvation.

Attention must also be called to the fact that the susceptibility method does not, as Lund asserts, depend upon the assumption that cyanide is a specific inhibitor of oxidations, for the regional differences in susceptibility, the susceptibility gradients as well as the differences with age and other conditions, are the same with many other chemical and physical agents and conditions in proper concentration or intensity, including for example high and low temperatures and lack of oxygen and perhaps external agents and conditions in general, as they are with cyanide.

The anesthetics and dyes used in the study of susceptibility in hydra, for example, give the same results as cyanide except where special irritating or paralyzing effects of those agents are concerned, and the modifications in susceptibility only serve to show all the more clearly the close relation between susceptibility and metabolic condition.

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[1] Lund was also informed in personal correspondence concerning our results on *Planaria* and the reason for the apparent discrepancy between susceptibility and total metabolism was pointed out to him.

The dyes were used in these experiments simply as a means of further analysis of the action of external agents. Since they possess color, it is possible to observe directly their penetration into the cells and to see that they enter all cells readily. It is certain that neutral red and methylene blue kill primarily from within the cell rather than by destroying or altering its surface first as perhaps some other agents do. They are adsorbed or otherwise accumulated by certain constituents of the cell, and it is of great interest to find that the general susceptibility relations with these dyes are essentially similar to these with other agents, except where special irritating or other effects are concerned. The dyes enter all cells, but they kill certain cells earlier than others because the cells differ in some way in physiological condition. Judging from their irritating effect, they probably increase metabolism to a very marked degree, at least at first, yet where the irritation is not too extreme, the death gradient is the same as in cyanide and other agents. No assumption concerning the specific action of cyanide is necessary as a basis for the susceptibility method, though it is quite true that the conception of the action of cyanide current among physiologists has been adopted and applied in connection with the method. The value of the susceptibility method rests not upon assumption but upon certain facts of observation and experiment: first, that in the susceptibility of protoplasm to many, perhaps to all, external agents a certain uniformity of relation appears as regards region of body, stage of life cycle, nutritive condition, etc.; and second, that this uniformity is in some way associated with and dependent upon the fundamental metabolic processes in the protoplasm and may be altered by changes in the rate of these processes.

Both E. J. Lund in his paper on starvation in *Paramecium* ('18*c*) and B. L. Lund in a paper on the susceptibility of *Paramecium* and *Didinium* to KNC ('18), which confirms earlier work of one of us (Child, '14*b*), maintain that susceptibility is dependent upon permeability and that permeability is independent of rate of oxidation. The chief basis for this view is the work of E. J. Lund on starvation, and it has been pointed out that his conclusions depend upon an interpretation of his data which in the light of the known facts is almost certainly incorrect: if this is the case, the whole contention of these authors fails. There are, moreover, certain data on susceptibility, such, for example, as the results with the dyes in hydra and other forms, which cannot be interpreted in terms of permeability in any physical sense, and other data on susceptibility to lack of oxygen, which show that other factors than permeability are concerned in susceptibility, are soon to appear. As one of us has stated elsewhere (Child, '19), the most important question in this connection is the question of the nature of what current terminology calls permeability. Extended discussion of this point is postponed to another time, but it may be noted that no purely physical conception of permeability accounts for all the known facts, that according to all the evidence, the surface as well as the interior of the cell is alive and the seat of metabolic reaction, and that susceptibility, and even permeability itself, are dependent, not merely upon the physical characteristics of the surface layers as a membrane, but also, at least in part, upon this living, chemically active condition. Apparently for the Lunds permeability is some condition at the cell surface completely dissociable from the metabolic condition of the protoplasm, but their own evidence that such a condition exists is at least far from conclusive as we have shown, and other adequate evidence is difficult to find.

Summary.

1. A study of the susceptibility of three species of hydra, *H. viridissima* (*viridis*), *H. vulgaris* (*grisea*) and *H. oligactis* (*fusca*) to various agents shows that a gradient in susceptibility exists along the apico-basal axis of the body and in each tentacle.

2. This susceptibility gradient is primarily a basipetal gradient, *i. e.*, the susceptibility decreases from the apical region basipetally and after the tentacles arise, in each tentacle from the tip to the base. This primary gradient appears in the earlier bud

stages, in quiescent attached individuals of *H. viridissima* and *H. vulgaris* at all stages and in young quiescent individuals of *H. oligactis*.

3. This primary gradient is modified in various ways by regional differences in functional activity and particularly by the special activities of the stalk, a more or less specialized organ which develops at a relatively late stage from the basal body region.

4. Regional differences in the contractile activity of the muscular apparatus produce the most conspicuous modifications of the susceptibility gradient. Such modifications can be produced by detachment of the animals, which leads to special activity of the stalk, by the general excitatory or special irritant action of certain agents, such as the dyes used, and by certain combinations of excitation and paralysis with anesthetics.

5. Alterations of the primary susceptibility relations are produced, not only by regional differences in muscular activity, but by regional differences in digestive activity.

6. The very direct and marked effect upon susceptibility of regional and local differences in muscular and other functional activity results from the simple and diffuse organization of hydra. The muscle, for example, is a part of the body cell and the whole cell shares in the excitation connected with muscular contraction, and the facts indicate that most or all local excitations spread or irradiate to a greater or less degree to adjoining regions.

7. In stages after the development of motor activity susceptibility decreases in general with advancing physiological age, but the bud in stages preceding the development of motor activity is less susceptible than later motile stages.

8. The differences in the susceptibility relations of the three species under various conditions suggest that of the three *H. oligactis* possesses, as its structural and functional characteristics indicate, the highest degree of regional specialization.

9. The facts confirm previous work in that they indicate the existence of a relation of some sort between susceptibility and metabolic rate. Within the ranges of concentration of the agents used, susceptibility evidently varies in general directly with metabolic rate, or with the rate of energy-liberating metabolism.

If such a relation exists, the axial gradient is a gradient in physiological condition, of which rate of fundamental metabolic reactions is one feature.

REFERENCES TO LITERATURE.

Child, C. M.

'13a Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. V., The Relation Between Resistance to Depressing Agents and Rate of Metabolism in *Planaria dorotocephala* and its Value as a Method of Investigation. Jour. Exp. Zoöl., XIV.

'13b Studies, etc. VI., The Nature of the Axial Gradients in *Planaria* and their Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwickelungsmech., XXXVII.

'14a Starvation, Rejuvenescence and Acclimation in *Planaria dorotocephala.* Arch. f. Entwickelungsmech., XXXVIII.

'14b The Axial Gradient in Ciliate Infusoria. BIOL. BULL., XXVI.

'15a Axial Gradients in the Early Development of the Starfish. Amer. Jour. Physiol., XXXVII.

'15b Senescence and Rejuvenescence. Chicago.

'15c Individuality in Organisms. Chicago.

'16a Axial Susceptibility Gradients in the Early Development of the Sea Urchin. BIOL. BULL., XXX.

'16b Axial Susceptibility Gradients in Algæ. Bot. Gaz., LXII.

'16c Further Observations on Axial Susceptibility Gradients in Algae. BIOL. BULL., XXXI.

'17 Susceptibility Gradients in the Hairs of Certain Marine Algæ. BIOL. BULL., XXXII.

'19 Demonstration of the Axial Gradients by Means of Potassium Permanganate. BIOL. BULL., XXXVI.

Hyman, L. H.

'16 An Analysis of the Process of Regeneration in Certain Microdrilous Oligochetes. Jour. Exp. Zoöl., XX.

'17 Metabolic Gradients in *Amœba* and their Relation to the Mechanism of Amœboid Movement. Jour. Exp. Zoöl., XXIV.

Lund, Barbara L.

'18 The Toxic Action of KCN and its Relation to the State of Nutrition and Age of the Cell as Shown by *Paramecium* and *Didinium.* BIOL. BULL., XXXV.

Lund, E. J.

'18a The Rate of Intracellular Oxidation in *Paramecium caudatum* and its Relation to the Toxic Action of KNC. Anat. Rec., XIV., Proc. Am. Soc. Zoöl., 1917.

'18b Quantitative Studies of Intracellular Respiration. II., The Rate of Oxidations in *Paramecium caudatum* and its Independence of the Toxic Action of KNC. Amer. Jour. Physiol., XLV.

'18c Quantitative Studies on Intracellular Respiration. III., Relation of the State of Nutrition of *Paramecium* to the Rate of Intracellular Oxidation. Amer. Jour. Physiol., XLVII.

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PRECIPITATION-STRUCTURES SIMULATING ORGANIC GROWTH. II.

A CONTRIBUTION TO THE PHYSICO-CHEMICAL ANALYSIS OF GROWTH AND HEREDITY.

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PART I. THEORETICAL.

The problem of growth, reduced to its simplest terms, is the problem of the conditions under which structure of a definite and specific kind is built up by the growing system through the chemical and physical transformation of material taken from the surroundings. As thus expressed, our definition applies to inorganic as well as to organic growth, *e. g.*, to the formation of a crystal from its "mother-liquid," or of a metallic deposit at a cathode. Both of these processes, especially the latter, exhibit many significant analogies to organic growth-processes; thus a crystalline or electrolytic deposit of a given chemical composition, laid down under constant external conditions, has, like an organic growth, its own definite and specific structural peculiarities. In organic growth and development, however, numerous complexities enter which are absent from inorganic growth; in particular the continual chemical and physical activity of the living system is always present as a dominating factor; this activity is itself specific and modifies in a specific manner the structure-forming processes, and is itself modified by them. Since every living organism is by its very nature an active system of this kind, the problem of organic growth becomes one relating not merely to the origination of specific *structure* but of specific physiological processes and activities as well, some of which are demonstrably dependent upon the observed structure, while others are related to it in a manner which cannot be precisely defined at present. Evidently structure, as such, in the sense of fixed disposition of material parts, represents only one side of the vital organization; its other and more characteristic side is manifested in the various special organic processes and the external behavior of the organism; these in turn imply the regulated concurrence, interaction and sequence of numerous simpler processes and events of a purely physico-chemical nature.

It is necessary, therefore, in forming a conception of the vital organization, to include as elements *all* of those constant or regularly recurrent features, both of structure and of activity, which contribute to the persistence or stability of the organism, considered as an individualized part of nature—*i. e.*, as a system in equilibrium with an environment. Any living being represents an integration or unification not merely of static conditions like structure, but also of *processes*, both simultaneous and successive; *i. e.*, the organism is to be considered not simply as a physical whole which is complete at any given moment, but rather as in its essential nature an ordered or regulated *sequence* of changes and activities, coördinated in space and time. For example, the succession of formative and physiological processes constituting development, or any special instinct requiring time for its exercise, is just as constant and characteristic a feature of the organization of a species as is its skeletal structure. What is most remarkable in the organism is the exact coördination of the various single activities, widely separated as they often are both spatially and temporally. Simultaneity in certain processes, ordered succession in others, are equally essential to its stable working; to be concrete, the normal activity of the heart and circulation in higher animals requires the simultaneous and coördinated activity of the nervous system, and the process of digestion depends upon the constancy of a certain definite succession of events in the alimentary tract and its related organs. All of this must be remembered in considering the problem of organic growth; we have to account for the construction of a system which is complex and specific in its *activities*, as well as in its *structure*.

It will be agreed that the possibility of any special organic function, consisting, as it typically does, in a definite and regular succession of simpler processes, depends upon the presence of a fixed and stable structural organization; without this there can be no organization of processes, *i. e.*, physiological organization, any more than there can be organized or coördinated activity without coördinated structure in an artificial mechanism like a locomotive. In this sense structure is of primary importance in any organism; the distinctive features of vital organization must therefore be referred ultimately to special structural peculiarities. This is obviously true of the various physical mechanisms and devices subserving particular functions (like locomotion, vision, circulation of blood, etc.) in higher animals; but it is the less familiar application of this principle to the general conditions of organic growth that I wish to consider in the present paper.

The structural conditions present at a given time in a growing or developing organism determine its present and future possibilities of growth. In a certain sense this is already well recognized; a definite structure can be observed in many instances in the germ before development, and certain features of this structure, especially the symmetry about certain axes and the distribution of certain materials, foreshadow the general morphological plan of the adult organism.[1] But in its details the structure of the egg is entirely different from that of the adult, and its precise relation to the latter must be regarded as unknown; *i. e.*, we cannot say definitely why in any species the transformation of the germ and of the material which it incorporates from the surroundings follows a constant and definite course, leading to a definite final or adult stage. Since, however, the building-up of organic structure from food and other materials is obviously a matter of constructive metabolism, any physicochemical theory of development must assume that a main factor in the formative process is the influence of the structural conditions already present upon the metabolic transformations by which more structure is formed.[1] At each stage of development the structure already present determines the nature of the structure which is being formed at the time; *i. e.*, the formative metabolism, like metabolism in general, is controlled by the structural conditions present in the growing regions, at the site of the syntheses and other chemical reactions concerned in the constructive process.[2] In considering the general physiology of growth the first question to be asked relates to the manner in which structure of a certain kind determines the production of further structure of the same or similar kind. Differentiation in ontogeny presents certain additional problems, but all developmental processes presuppose *growth*, or increase in organized material of a type already present.[3]

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[1] For a general account of the known correspondences between the structure of the egg and that of the adult, *cf.* Conklin's recent book, "Heredity and Environment in the Development of Man," Princeton University Press, 1918; also his recent paper, "The Share of Egg and Sperm in Heredity," *Proc. Nat. Acad. Sci.*, 1917, Vol. 3, p. 101.

It is to be assumed that the chemical reactions constituting the specific metabolism of any cell or other living system are under the control of structural conditions of a highly definite kind. Protoplasm is polyphasic in its physico-chemical constitution, and both the nature and the velocity of the chemical reactions in such a system must be largely influenced by boundary conditions (adsorption, capillarity, interfacial potentials, etc.); hence in a system of a definite chemical composition constancy in the arrangement, character and extent of the phase-boundaries, *i. e.*, in the *structural* conditions present in the reacting system, implies corresponding constancy in the reactions taking place under such influence. In other words, if the structural conditions are definite, the chemical processes whose rate, nature and position they control must also be definite; and the products of these reactions, if they are of such a nature as to accumulate and add to the structural material of the system, will also be definite and will be deposited in definite situations and at definite rates and times. Apparently deposition of this kind forms an essential part of the organic growth-process.[1] Any structural material thus added will secondarily influence further growth, and by degrees, if metabolic activity continues, a definite structure will be built up whose precise nature, when the final stage is reached, will have been determined by the structure originally present in the growing system—assuming constancy in the original chemical composition of the latter, and in the conditions to which it has been exposed during its growth.

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[1] The general features of this relation are very clearly discussed in Child's "Senescence and Rejuvenescence," University of Chicago Press, 1915, Chapter 1, pp. 26 *seq.*; *cf.* also the special papers there cited.

[2] It should be noted that the site of the structure-producing chemical changes may in some cases be *extracellular;* materials produced by normal metabolism within the cell may pass to the exterior (be secreted, etc.) and later undergo chemical change giving rise to definite structure; supporting or skeletal structures are often thus formed (matrix of bone, cartilage, connective tissue, etc.); other instances are formation of spiders' webs and similar structures, clotting of blood, etc.

[3] In differentiation, *i. e.*, the general process by which groups of descendants of the same germ-cell become structurally and physiologically different and functionally specialized in later development, factors seem to enter which are absent in simple growth. The phenomena of Mendelian inheritance plainly suggest that differentiation is controlled by the distribution of a limited number of structure-determining factors embodied in chemically stable material particles which can be transferred with properties intact from cell to cell. The chromosomes answer to this conception, and apparently form an essential part of the special physiological mechanism controlling differentiation; of course this control must be exercised by modifying metabolism, in a manner and by means which are as yet unknown.

In any living organism the characteristic physiological activities and external behavior must have as their basis a constancy and specificity in the chemical and physical conditions underlying the fundamental vital processes. These conditions are in large part structural; if we could account fully for specific structure, we could not doubt account also for what is specific in physiological activity and behavior. Now specific structure is not confined to organisms; it is perhaps most perfectly exemplified in the specific form of crystals; and crystalline form is to be referred in turn to the chemical character and mode of arrange- of the molecules composing the crystal. In attempting to account for the specific form-characters of organisms we must first inquire, as a matter of general scientific procedure, whether it is not possible to refer these to the more general conditions present in other natural systems which also exhibit definite and specific form-characters. The case of crystals is the clearest; here the visible structure is related to the structure of the molecules and varies with changes in the nature and relative positions of the atoms; this relation of molecular configuration to crystalline form is best illustrated by the differences in the right and left-handed crystals of stereoisomeric compounds like the tartrates. In the chief compounds forming the structural basis of living protoplasm, the proteins, we have molecules of great complexity, consisting essentially of chains of asymmetric molecules united by an easily dissoluble linkage; it is this structure which renders possible the high degree of specificity shown by the individual proteins. There is definite evidence that to this chemical specificity corresponds an equally definite morphological or structural specificity in the crystalline or other aggregates formed by proteins when they separate out from solution. Reichert and Brown's observations indicate that if our knowledge were sufficiently exact and detailed different species could be distinguished and identified by the crystal-forms of their hæmoglobins,[1] just as birds can be distinguished by their feathers; and presumably similar relations between specific molecular structure and the structure of solid aggregates hold true for homologous proteins of other kinds. Probably it is to the intimately or microscopically crystalline nature of the deposits that the possibility of distinguishing different protein precipitates by their microscopic characters is due.[2] According to von Weimarn all solid material, including matter in the "amorphous" and colloidal states, is ultimately crystalline in structure;[1] and this general conception is in conformity with the more recent evidence from other departments of physics indicating that the molecules in solid matter are oriented regularly to form definite geometrical patterns (space-lattices).[2] If therefore to a specific molecular structure there corresponds a specific *molar* structure (or structure peculiar to the substance in its solid state of aggregation), it follows that any specific protein which is synthesized and laid down in solid form within the cell, or at the cell-surface or in any other situation, will necessarily produce a definite and specific type of structure different from that formed by any other protein. Such a chemical synthesis would thus involve a morphological synthesis of a simple kind; to the constant structure of the single molecules would correspond certain constant form-characters in the larger aggregates built up from these molecules. It seems clear, therefore, that if specific proteins are synthesized in any organism, and if

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[1] Compare the interesting discussion of Child, *loc. cit.*, Chapter 2, pp. 38 *seq.*

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[1] *Cf.* Reichert's very suggestive article, "the Germ-plasm as a Stereochemic System," *Science*, 1914, N.S., Vol. 40, p. 649. Reichert also regards heredity as ultimately an expression of the influence of the specific structural characters of the cell-proteins upon the course of formative metabolism. "Given as the basis of scientific study a germ-plasm that has inherently the power of development; that is in the form of a stereochemic system that is peculiar to the organism; that is highly impressionable to stimuli; and that has the marked plasticity that is inherent to organic colloidal matter, we have all the postulates that are needed as a foundation upon which, according to the laws of physical chemistry, can be built a logical explanation of the essential fundamental elements of the mechanism of heredity . . . The typical condition of matter of definite composition is crystalline. . . . Having a homogeneous solution of various selected crystalline substances of appropriate chemical composition and constitution, and given conditions attendant to crystallization, the successive stages of crystalline development will proceed along fixed and definitely recognized lines. . . . Having in the germ-plasm an analogous physico-chemical system, . . . the phenomena of development likewise proceed in conformity with the same laws along definite lines . . . " (pp. 656–7). These quotations will indicate Reichert's general point of view. His conception of the manner in which this specific structure influences metabolism and thus determines further structure-formation is, however, different from that outlined in the present paper.

[2] This seems possible to a considerable degree. Alfred Fischer's work, "Fixierung, Färbung und Bau des Protoplasmas," Jena, 1899, Chapters 3–5, represents a beginning in this direction; further development of this technique seems desirable.

Della Valle's view that the form-characters of individual chromosomes are an index of a definite microcrystalline structure is especially interesting, since in this case a specific chemical composition of each chromosome would be implied by its specific and constant form-characters. *Cf.* Della Valle: *Archivio Zoologico Italiano*, 1912, Vol. 6, p. 37; also *Zeitschr. f. Chemie u. Industrie d. Kolloide*, 1913, Vol. 12, p. 12.

[1] Von Weimarn's observations and theoretical discussions are chiefly published in *Zeitschrift für Chemie und Industrie der Kolloide*, 1907–13, Vols. 2—13, though partly in Russian journals. The work is too extensive to be summarized briefly, but its experimental part consists largely in showing that all transitions between typically colloidal systems of inorganic and organic compounds and definitely crystalline systems may be obtained by varying the conditions of formation of the compounds, *e. g.*, the concentrations of the solutions (*e. g.*, $Ba(CNS)_2$ and $MnSO_4$) mixed to form the compound in question, the solubility of the latter, its rate of formation, etc. In this manner the crystalline structure of gels and other colloidal systems is clearly indicated. Protein gels are thus crystalline in the microscopic or submicroscopic character of their solid phase, and hence possess a specific inner structure. The crystalline nature of fibrin gels has recently been clearly shown by Howell; *cf. Amer. Journ. Physiol.*, 1914, Vol. 35, p. 143, and 1916, Vol. 40, p. 526. For the microcrystalline structure of inorganic precipitation-membranes *cf.* von Weimarn, *loc. cit.*, 1908, Vol. 2, pp. 280 *seq.*, and photographs there reproduced.

[2] *Cf.* the papers by W. H. and W. L. Bragg on the patterns produced by the diffraction of X-rays at the surfaces of crystals, indicating a space-lattice arrangement of the atoms: *Proc. Roy. Soc.*, 1912, Vol. 88 A, p. 428, and 1913, Vol. 89 A, pp. 248, 277, 430, 468.

their molecules segregate and the compounds separate out in solid form, specific structure will be the inevitable result. And this structure, having once arisen, will exert its own specific influence upon the ensuing chemical and physical transformations in the system. Any metabolism thus controlled by specific structure must exhibit specific features, and if it produces further structure this will also be specific, and will influence specifically the succeeding metabolic processes by which more structure is formed.[1]

If we consider the growth and development of the germ in the light of these general considerations, we are led irresistibly to the conclusion that in any specific sequence of form-changes, such as those constituting development in any organic species, the character of the formative transformation proceeding at any stage, as well as the special character of the terminal stage of the whole sequence, the adult organism, must be determined by the original structure of the system—that with which the growth begins. It is clear that the conditions, structural and other, present when growth is *begun* must affect the entire succeeding series of transformations. The initial factors, however, are not the only ones to be considered; changes in external conditions appearing at any time during the series must contribute their influence; and the eventual outcome will be determined not only by the initial state of the growing system and its surroundings, but also by the conditions present at any time during the process of growth. This conclusion clearly implies that if the surrounding conditions are kept *constant*, a growing system of a given initial constitution will undergo a series of transformations which will also be constant, *i. e.*, definite or predetermined. The whole developmental sequence may then be said to have been *determined* by the original constitution of the system; that is to say, two or more such similar systems, placed under the same external conditions, will develop in the same way and will reach similar final stages at the same time. Conversely, two systems *differing* in initial constitution, placed under similar external conditions, will be transformed in different manners and at different rates and will form different final products. The case of two eggs of the *same* species developing side by side in sea-water illustrates the first case; that of two eggs of *different* species the second. The corollary follows, that any attempt to account causally for the special structure or activities of an organism at any stage, adult or embryonic, must always involve a *regressus* to the original conditions with which development starts. One is thus inevitably brought back to the organization of the germ as a starting point.[1]

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[1] Obviously any chemical synthesis involves a morphological synthesis, in the sense of producing molecules with a definite spatial distribution of atoms and hence a definite geometric form or configuration. But it is only when these molecules unite or segregate (with axes parallel) to form solid structures like crystals or crystal-aggregates, that the chemical synthesis becomes the condition of morphological synthesis in the crystallographic sense; the vectorially acting interatomic forces determine the special configuration of the molecule, and when union occurs to form large aggregates the latter exhibit specific geometrical characters which are determined by those of the individual molecules. The present contention is simply that morphological synthesis in organisms is also ultimately determined, in the somewhat indirect manner indicated above, by this structural specificity of the solid material produced in metabolism. A definite internal structure is thus imparted to protoplasm, and this determines a definite and specific formative as well as other metabolism.

The general conclusion follows: any germ with a definite or fixed physico-chemical organization, implying constancy in the composition, distribution, and physical state of its structural and other chemical components, which is in process of incorporating materials from the surroundings (or its own yolk reserves) and transforming these materials so as to form further structure, will necessarily follow a constant course of transformation if the external conditions are also constant; this will apply both to the type of structure making its appearance at any stage, and to the physiological processes and other activities whose nature depends on that structure. Apparently we have here the general type of situation presented by the developing germ or other living system in process of growth. Given a specific organization at the outset, together with constancy of conditions, external and internal, under which the incorporated material is transformed and accumulated, and the system will undergo a cycle of chemical and physical transformation which will itself be constant and specific. That is, two or more such systems will undergo identical transformations. This, put simply, appears to be the general property or condition which in living organisms we call "heredity."

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[1] The origin of this organization is of course similarly to be referred to preëxisting conditions, and strictly speaking any such *regressus* is *ad infinitum*. In any sequence of physical processes each stage is at once conditioned by the preceding and the condition of succeeding stages—*i. e.*, is both determined and determinant. For an admirable discussion of the relation of this feature of reality to developmental processes in general, *cf.* Hobhouse's "Development and Purpose," Part 2, especially chapters 4 and 5.

The artificial growing systems considered in the present and preceding papers exhibit in a simplified form the same general type of behavior. We have seen that in a system of a given composition chemical reactions occurring under structural conditions of a definite kind will give rise to definite reaction-products at a definite rate and in definite situations; hence if solid—*i. e.*, structurally coherent and stable—material is among these reaction-products, this material as it accumulates will add to the already existing structure in the system and will itself influence in a definite manner the succeeding reactions and structure-formation. In the formation of precipitation-structures or crystallization-structures by combinations of mutually precipitating dissolved salts this general type of phenomenon is exemplified in a simple manner; and when the precipitates have certain physical properties—low solubility, fine subdivision, colloidal quality, coherence—the structures thus formed often exhibit a close resemblance to simple types of organic growth. Precipitation-structures of copper ferrocyanide, made by running a solution of copper sulphate beneath a solution of potassium ferrocyanide, have long been used to illustrate this phenomenon. The essential basis for this resemblance is undoubtedly the formation of semi-permeable membranes by the precipitate as it forms; these, by preventing diffusion, furnish the conditions for the osmotic entrance of water into the various membrane-enclosed spaces (which are typically of a vesicular, cellular or tubular form); a more or less definite localization of osmotic processes and of the direction of flow of solution results; hence the deposition of precipitate is also localized and a correspondingly definite structure is formed; which resembles that of simple vegetative growths because of the importance of the osmotic factor in both cases. The size, shape, and other characters of the structures formed under these simple conditions are not so constant as in the majority of organic growths; but much deviation from type is to be expected when accidental variables are not compensated or excluded—as they typically are in the delicately regulated growth-processes of living organisms. Even in organisms, however, the degree of form-regulation varies, and in many plants especially the "habit" of growth is not fixed but changes with the conditions. Precipitation-structures formed according to the directions of Leduc or Herrera from a prescribed combination of materials[1] exhibit many morphological features which are constant and determined mainly by the chemical nature of the structural material. Similarly in the precipitation-structures formed from metals by electrolytic local action, as described in the present and preceding papers, the tubules and vesicles of *iron* ferricyanide are characteristically different in appearance and structure from those of (*e. g.*) *zinc* ferricyanide formed under the same external conditions; in fact each metal (Fe, Co, Ni, Zn, Cd, Cu) forms a specific and easily recognizable type of precipitation-structure. The chemical nature of the structural material—insoluble precipitate of inorganic salt in this case—determines the special form-characters assumed by the single filaments, vesicles and other structures which grow out from the metal; and if these structures are allowed to accumulate until conditions of equilibrium are reached and growth ceases, the whole resulting mass of precipitation-formation is then found to present an appearance of a constant and characteristic kind (see photographs, Plates 1–6).

In the analogous case of the living organism, *e. g.*, a higher animal, where a definite and final form and structure are attained at the end of a long period of development and growth, it is known that the structural material has a constant and highly specific chemical composition; and it is to be assumed that this composition has a determinative relation to the physical and other properties of the organized structure thus arising. In this respect the two types of growing system under comparison exhibit certain fundamental similarities in the general physicochemical nature of the formative process; and it seems desirable to recognize these resemblances and to formulate them clearly (without of course ignoring or underestimating the many and obvious differences). In both the living and the non-living growing systems the structural material is the product of chemical reactions whose rate, character, and position depend upon the chemical nature of the interacting substances and upon the structural conditions under which the reactions take place. In the living system, however, on account of the minutely graded chemical and structural specificity and the exact regulation of the conditions under which the metabolic and structural transformations occur, the constancy or determinateness of form, structure, and activity when the final or adult stage is reached is much greater than in the inorganic system; and it is only in its broadest and most general features that the growth of the latter can be regarded as a model for that of a living organism. Indeed the most astonishing feature of organisms has always been that from a minute germ a system of the utmost chemical and physical complexity, yet identical in all respects with another system arising similarly from another germ of the same kind, should be built up by the chemical transformation of materials taken from the surroundings. Nevertheless something of the same kind is seen in the building-up of a precipitation-structure under the influence of a piece of metal placed in a ferricyanide solution. Here also the material of metal and solution is chemically and structurally transformed in a constant manner, and a system of definite physico-chemical constitution and activity is formed whose peculiarities of structure and activity have a specific dependence upon the nature of the introduced metal.

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[1] *Cf.* Leduc, "Mechanism of Life," London, Rebman Ltd., 1911; Herrera, *Boletin de la Direcion de Estudios Biologicos, Mexico*, Vols. 1 and 2, 1916 and 1917.

The precise relation between the specific chemical constitution of the proteins of the living germ—*i. e.*, of those structural compounds which are demonstrably peculiar to the species in question—and the specific type of structure arising in growth or development is unknown. It must be recognized that other factors than the chemical specificity and arrangement of the structural proteins play a part in determining the precise course of the formative process; *e. g.*, changing the nature and proportions of the salts of sea-water produces definite modifications of development in the eggs of marine animals; well-known examples of this effect are the cyclopia induced in fishes by adding magnesium salts,[1] and the production of exogastrulæ in sea-urchin eggs by lithium chloride.[2] The germinal protoplasm contains inorganic salts and numerous other non-specific compounds, differing in their nature, concentration, and distribution in different germs; moreover the catalysers present and the various purely physical peculiarities of the system (*e. g.*, viscosity, capillarity, boundary-potentials, permeability to diffusing substances, etc.) must all enter as factors influencing the rate and character of the chemical and physical processes concerned in development. All such factors must be included under the conception of *germinal constitution;* but we are still far from understanding why a given germinal constitution determines in such minute detail the course of the proliferative and transformative process constituting development in any particular species. It seems certain, however, that chemical specificity is the primary and fundamental factor. All organic structure is built up by chemical processes, *i. e.*, by metabolism; hence the divergences first appearing in evolution between different species must have been primarily *chemical* in nature, since they obviously involved—or consisted in—divergences in the character of the specific metabolic syntheses on which the growth, continued life and special character of any organism depend. The essential differences in the germinal constitutions of different species must similarly depend on specific chemical differences, *i. e.*, primarily on the specific constitution of the proteins; these differences will determine specific structural differences, as we have seen. For example, it seems probable that the egg of the starfish and that of the sand-dollar—which are closely alike in general appearance and properties—are not widely dissimilar in their purely chemical constitution, except as regards the specific nature of their structural proteins; and that it is ultimately because of this difference that the two germs effect such different transformations in the food and other materials which each incorporates from the surroundings and builds up into an adult organism of constant type.

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[1] Stockard, *Archiv f. Entwicklungsmech.*, 1907, Vol. 23, p. 249; *Journ. Exper. Zoöl.*, 1909, Vol. 6, p. 285.

[2] Herbst, *Zeitschr. wiss. Zoöl.*, 1892, Vol. 4, p. 446; *Mitt. zoöl. Sta. Neapel*, 1893, Vol. 11, p. 136.

But a detailed knowledge of the constitution of these proteins, *i. e.*, of the chemical nature and serial (or other) arrangement of the amino-acids united to form the protein molecule, would not at present—if ever—enable us to predict the course of development of either egg.

We may assume, however, that the essential reason for such a ailure would be that the conditions in the living system are too *complex* (for historical and other reasons) to be analyzable in the requisite detail, and not that a sufficiently complete knowledge of the initial state of the system would never enable us to predict the general course and outcome of the developmental transformation. Under these circumstances the study of some relatively *simple* inorganic process, which in certain respects may serve as a model for the organic formative process, may throw light upon the general features of such a problem and indicate the type of solution which it is practicable to seek. The purely scientific aim is not to follow the developmental sequence through all of its stages and account causally for each separate transformation, but rather to determine what the essential and constantly operative factors are in *all* cases of development,—*i. e.*, the *general* conditions which make possible a specific type of development in any particular case. Each case presents its own special problems; but there are factors common to all cases of development, and any general theory of development requires that the nature of these should first of all be clearly indicated.

In the inorganic growth-structures described in the present and preceding papers[1] certain definite relations can be recognized between the type of structure formed and the chemical and physical character of the precipitate which is deposited to form that structure. Thus filaments of zinc ferricyanide are coarser and less coherent, as well as more variable and irregular in form, than filaments of iron ferricyanide formed in the same solution (see Figs. 1–5, 12–15). The metal may be compared with the germ; its specific chemical nature determines the special type of structure to which it gives rise in a given solution of potassium ferricyanide—*i. e.*, under definite external conditions; the reason for this is that each insoluble ferricyanide has its own special physical and structural peculiarities which vary with the nature of the heavy metal. The observed structural difference between these two kinds of filaments appears to be largely determined by the difference in the physical nature and consistency of the precipitate; this is more finely divided and coherent with iron than with zinc, and greater smoothness of contour and uniformity of structure in the growing filaments are the result. Again, the two closely related metals, nickel and cobalt, whose ferricyanides have almost identical physical properties, illustrate the same principle in a highly characteristic manner; the precipitation-structures formed from these two metals are strikingly similar in appearance, exhibiting what might be described as a close family resemblance, *e. g.*, in the coarse texture and opacity of the filaments and in the characteristically winding and tortuous course which they both adopt (see Figs. 16, 17, 21); this latter peculiarity is found in none of the filaments formed from other metals. Similarly zinc and cadmium form filaments and vesicular formations (Figs. 12, 13, 18, 19) which are closely alike in structure and appearance.

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[1] R. S. Lillie, Biol. Bull., 1917, Vol. 33, p. 135.

It is interesting to note that this resemblance extends not only to the form-characters of the completed structure but also to the peculiar type of physical activity which it exhibits during its growth. The precipitate forming the walls of the striated vesicular or shell-like structures characteristic of zinc and cadmium (see Figs. 13, 18, 19) is deposited rhythmically or intermittently; the striation is a direct consequence of this rhythm, which itself is due to an intermittency in the outflow of the solution from the interior of the growing vesicle; this intermittency depends upon the alternating formation and rupture of the precipitation-membrane forming the wall of the vesicle. The rate of this rhythm, and hence the rate at which the precipitate is deposited and the resultant character of the striation, are similar in the two metals. Evidently these peculiarities of behavior are to be referred ultimately to the special physico-chemical properties of the precipitate and of the metal; zinc and cadmium, being chemically closely related metals, form ferricyanides having similar properties; accordingly they give rise to structurally similar deposits, which under similar external conditions exhibit similar behavior. It should further be noted that this behavior is itself a factor in the determination of the type of structure formed. The structure of the system and its physical activity thus mutually influence each other, and the eventual outcome in permanent structure deposited is the result of this continual interplay of chemical and physical factors.

The whole sequence of events in the formation of a single striated precipitation-vesicle of cadmium ferricyanide may be briefly sketched as follows (for further details see below, page 260). The original vesicle or tubule with which growth starts is formed at an anodal region on the surface of the metal; here cadmium ions enter solution and interact with ferricyanide anions to form cadmium ferricyanide, which is deposited as a thin easily ruptured semipermeable precipitation-membrane. The interior of this original precipitation-vesicle contains a soluble salt of the metal (*e. g.*, $CdCl_2$, formed from soluble chloride in the solution), and more precipitate is formed wherever the wall of the vesicle ruptures and the solution flows out from the interior to meet the potassium ferricyanide outside. The direction and rate of this outflow depend upon various physical factors (form and size of vesicle, character of local circuit, external conditions, etc.), which consequently determine where and at what rate new precipitation-structure is formed. The physical properties of the newly deposited precipitation-membrane, which by degrees builds up the wall of the growing vesicle, depend upon the specific chemical nature of the precipitate (since to a definite chemical composition correspond definite physical properties); these determine the properties of the wall of the vesicle, *e. g.*, its elasticity, coherence and resistance to rupture, and hence the rate of the rhythmical rupture; this again determines the rhythm in the outflow of solution, and consequently the rhythm of precipitation; and this latter rhythm determines the arrangement and spacing of the striations, *i. e.*, the characteristic morphological features of the completed structure.

This whole sequence may serve as a simplified model or epitome of what takes place in any organic formative process. In both the living system and its inorganic model the energy for the work of growth is derived from the energy freed in chemical reactions. Certain insoluble and physically coherent products of these reactions form structure, which as soon as formed influences in a definite manner the chemical and physical processes by which further structure is formed. In its special character this structure varies with the chemical nature of the structural material; as is general with matter in the solid form, this material is deposited from solution in a crystalline or quasi-crystalline state of aggregation, which is structurally specific in the same sense as the molecular structure is specific. Thus the microscopic and ultimately the macroscopic form-characters of the growing system are determined by the special molecular structure of the formative material.

Organic growth, as Child has so clearly pointed out, depends upon the progressive accumulation of the solid metabolic products which are stable under the conditions. Since the chief of these structure-forming products, the proteins, have the most minute and detailed specificity known among chemical compounds, it is not surprising that the living systems built up from these compounds should be correspondingly individualized and specific in their structure and activities. The formation of precipitation-structures from metals may be regarded as a simplified model-process which makes it easier to understand why organisms whose proteins are similar in chemical configuration (as shown *e. g.* by precipitin tests) are also structurally and physiologically similar—*i. e.*, are closely related in the natural system. Chemical similarity and structural similarity go hand in hand, and structural similarity involves similarity in physiological activity and external behavior.

We have seen that the formation and activity of these precipitation-growths are the result of processes of electrolysis in the local electrical circuits arising between different areas of the metallic surface. The currents of these circuits control the structure-forming process; and in conformity with this condition we find experimentally that the formation of filaments can be promoted, modified, or suppressed at will by electrical means.[1] In this respect also an analogy to organic processes is to be seen. The electrical sensitivity of living matter is one of its most

[1] R. S. Lillie, *loc. cit.*, pp. 148–157.

characteristic peculiarities; and since the energy of any vital activity resulting from electrical stimulation—*e. g.*, muscular movement—is derived from chemical change in the tissue, it is clear that this sensitivity implies an influence of the electric current upon the chemical or metabolic processes by which energy is freed. These processes are mainly oxidations, and the energy thus freed may be applied in chemical and structural synthesis, as well as in motor or other activity. The facts of electrical stimulation, inhibition, and directive control (*e. g.*, in galvanotropism) show that the chemical reactions in protoplasm can be initiated, altered in rate, or suppressed by the electric current; and there is much evidence that the electric currents produced by living cells in their own activity exert normally a controlling influence of this kind. In the two preceding papers of this series I have briefly reviewed the known facts indicating that the organic growth-processes both give rise to and are influenced by electric currents.[1] The universal susceptibility of organisms to electric stimulation is in itself indirect evidence that normal growth is subject to this form of control, since functional activity, which is readily initiated by the current, has a profound influence on growth-processes. Bioelectric currents appear always to accompany normal functional activity, and many of the most fundamental cell-processes, such as the transmission of states of excitation in cells and nerve-fibres, appear to be directly determined by the currents of local bioelectric circuits.[2] A dependence of functional activity upon bioelectric processes implies a dependence of growth upon such processes, although further investigation is needed before the precise nature of this interconnection can be defined.

The recent interesting work of Child and Miss Hyman[3] has given further direct evidence of such an interconnection; they find that the most actively growing zones in various animals, especially annelids and planarians, are electrically *negative* to other regions. The evidence now available indicates that this condition is a general one, and that in growing tissues and organisms (both animals and plants) the regions of preponderant growth are typically electronegative (in the physiological sense) to less actively growing regions, *i. e.*, at the actively growing regions the positive stream of the bioelectric circuit enters the living cells from the surroundings. It is well known that in irritable tissues the physiologically active or stimulated regions are negative to resting regions; growth appears to agree with other forms of functional activity in the character of its bioelectric manifestation. The fact that functional activity is in general a condition favorable to growth is probably to be connected with this fundamental similarity, and suggests that the bioelectric currents associated with activity have themselves a growth-furthering influence upon the tissue. Now organic growth implies the synthesis of specific compounds, especially of proteins; apparently therefore this synthesis is favored where the positive stream enters the protoplasmic surface from the outside. In the typical irritable tissue like muscle or nerve the bioelectric current leaves the irritable element at the resting regions and enters it at the active region; but activity at the latter region is usually temporary, unless stimulation is continued, and it is probable that the normal automatic return to the resting state is directly determined by the re-entrance of the current into the irritable element at the active area.[1] We know that a positive electrode from an external source of current has an inhibiting effect when applied to an active area of tissue, and the same effect must result at the entrance-region of the current generated by the tissue in its own activity.

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[1] *Loc. cit.*, p. 181; BIOL. BULL., 1918, Vol. 34, p. 85.

[2] *Cf.* my recent series of papers on the general physiology of protoplasmic transmission: *Amer. Journ. Physiol.*, 1914, Vol. 34, p. 414; 1915, Vol. 37, p. 348; 1916, Vol. 41, p. 126; *Science*, 1918, N. S., Vol. 48, p. 51; *Scientific Monthly*, 1919, Vol. 8, p. 456.

[3] *Cf.* L. H. Hyman, *Science*, 1918, N.S., Vol. 48, p. 518.

All of these facts receive a consistent unitary interpretation on the hypothesis that the entrance of the electric current (positive stream) into the protoplasm induces or promotes certain oxidation-processes, primarily in the surface-film, and that these oxidations form a necessary condition of the synthetic processes by which the specific structural material of protoplasm is built up. In the actively growing cell or organism this synthesis results in a permanent increase in the quantity of living substance. In the stimulated irritable element (*e. g.*, muscle-cell) growth is not always evident; but there is evidence that the permeability of the surface-film is increased during stimulation (this structure being apparently broken down or otherwise altered in the stimulation-process), and it seems probable that the normal semipermeable condition is restored by a synthetic process of the kind indicated, and that in this manner the cell regains the resting state. According to this view the altered surface-film is restored to its original or resting state after stimulation by a process involving both chemical and structural synthesis, which takes place under the influence of the local biolectric circuit. Presumably repair of the cell-surface after local injury is accomplished in an essentially similar manner. The need of oxygen for the processes of growth, regeneration, or recovery from poisoning, narcosis or over-stimulation (fatigue) may be understood on this general hypothesis; in all such cases oxidation-processes occurring under the control of local bioelectric circuits are necessary for the syntheses involved in the process of construction or restitution.[1]

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[1] The automatic repassivation of iron in strong HNO_3 offers a close physicochemical analogy; *cf.*. my paper in *Science, loc. cit.*, p. 55.

An instructive physico-chemical analogy is furnished by the local electrochemical reactions concerned in the transmission of the active state over the surface of passive metals, *e. g.*, iron,—a process apparently similar in many essential features to protoplasmic transmission.[2] Here the propagation of the local activation from region to region is directly due to local circuits formed at the boundary between the active and passive areas of the metallic surface; in an iron wire immersed in strong nitric acid the protective or passivating surface-film of oxidation-product is formed at one region of the surface (the anodal) as the activation-wave passes, and broken down by reduction at an adjoining region (cathodal). In such a wire any local interruption of the surface-film is thus at once automatically repaired, since that region, by the exposure of the underlying metal, is rendered anodal, and hence becomes the site of formation and deposition of the passivating surface-film. Similarly in the production of the local bioelectric circuit, *e. g.*, in a stimulated nerve, there is apparently a physical and chemical alteration of the protoplasmic surface-film, involving increased permeability, at one region, that of excitation; this altered region becomes electrically negative, so that the positive stream of the resulting bioelectric current there re-enters the protoplasmic surface; and apparently it there effects an oxidative synthesis which restores the resting condition. According to this conception, constructive or "anabolic" processes predominate at the regions where the current *enters* the protoplasm from outside; while at the adjoining inactive regions where the current *leaves*, and where a new state of excitation is automatically aroused, it is to be assumed that processes of the reverse kind take place.[1]

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[1] Loeb's observations on the breakdown of the cell-walls in the blastomeres of the *Ctenolabrus* egg in the absence of oxygen and their re-formation when oxygen is readmitted afford a striking instance of the dependence of structure-formation upon oxidation-processes. *Cf. Archiv. f.d. ges. Physiol.*, 1895, Vol. 62, p. 249; also in "Studies in General Physiology," University of Chicago Press, 1905, Vol. 1, p. 370.

[2] *Cf.* my discussion of this resemblance in *Science, loc. cit.*

It is interesting to note that many years ago, before the development of modern physical chemistry, Hering reached very similar conceptions of the relation of the electric current to stimulation and to vital activity in general.[2] He rejects Du Bois-Reymond's purely physical conception of the origin and significance of the bioelectric currents, and recognizes that they are essentially indexes of chemical changes in the living matter. Similarly, external electric currents affect living matter primarily through their *chemical* or metabolic influence; where the current enters the protoplasm *assimilatory* (anabolic) processes predominate, and where it leaves, *dissimilatory* (catabolic); the stimulating and electrotonic influences of the current are referred to these characteristic influences on metabolism. These conceptions are surprisingly in accord with our modern physico-chemical point of view. The physiological polarity in the action of the current implies a polar difference in the chemical effects which it produces in living matter. The closest physico-chemical analogy is electrolysis; and it is to be inferred that processes of the same essential nature as electrolysis underlie the chemical effects of the electric current in organisms. This possibility was partly recognized by the older electrophysiologists,[1] but in the absence of any clear conceptions of the fundamental physico-chemical constitution of living matter they could do no more than make this suggestion and trust to later investigation and criticism for confirmation or disproof.

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[1] Since such constructive processes are the condition of growth, we should expect, according to this hypothesis, that the constant electric current in influencing growth should *promote* the latter where the positive stream enters the tissue (*i.e.*, at the anode of the pair of applied electrodes) and *inhibit* where it leaves (at cathode). According to the recent observations of Bose (Proc. Roy. Soc., B, Vol. 9, p. 364) this is the case in plant growth. "In the polar action of electric current on growth, the anode is found to enhance and the cathode to depress the normal rate" (p. 399).

[2] Hering, "Theory of the Functions in Living Matter," *Brain*, 1897, Vol. 20, p. 232 (translation of article in *Lotos*, IX., Prag, 1888).

In the precipitation-structures described in this paper the new structural material is also formed at the anodal regions of the metal; and the inorganic growth-process at any region can be accelerated by making that region more strongly anodal, or inhibited by making it cathodal. The process of construction is thus susceptible to electrical influence, and this influence exhibits polar character—a necessary consequence of its dependence on electrolysis. Obviously the resemblance of the inorganic model to the living system relates only to the general nature of the conditions which control the chemical reactions underlying the formation and deposition of structural material. The detailed character of the formative processes in living matter can be made clear only by further investigation, but recognition of the general physico-chemical class to which the processes belong is essential as a guide in this investigation.

The senior author is responsible for Part I. of the present paper. The observations and photographs contained in Part II. were all made by Mr. Johnston, whose own account of his work now follows.

Part II. Experimental.

I. *Introduction.*

It is the purpose of this paper to describe in some detail various types of precipitation-structures produced by means of electrolytic local action in metals, according to a method de-

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[1] *Cf.* E. du Bois-Reymond, "Untersuchungen über thierische Electricität," Vol. 2, p. 387.

scribed in a previous paper by the senior author.[1] In the present paper description of methods will be confined to certain modifications which have been found to be more suitable to the production of a certain definite type of structure. Certain conditions determining the type of structure produced have been studied in fuller detail. Expecially interesting is the fact that the process of formation is often periodic and accompanied by rhythmic motion of the structures formed. Regularly segmented or striated formations are thus often produced. Many interesting biological comparisons are therefore suggested.[2]

II. *Methods.*

If a piece of fine iron wire, wound about one end with a fine copper wire, be dropped into a 2 per cent. egg-albumin solution containing 4 per cent. K_3FeCy_6 and 4 per cent. or more of NaCl, the entire surface of the iron wire rapidly becomes covered with fine filamentous growths (Fig. 1). They are characteristically regular in form; the majority are straight or slightly curved and cease growth at a length of 200 microns or less. Very few, if any, of the larger striated or branching structures are formed under these conditions. A repetition of this experiment using a less concentrated solution of the salts (2 per cent. egg albumin containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl) gives a different result. The rate of growth is decreased, the form of the filaments is more irregular and many larger structures are formed (Fig. 2). In the stronger solution growth usually ceases in about five minutes, while in the weaker solution it may continue for several hours.

One method of controlling the rate of growth is to start the reaction with a quantity of the solution just sufficient to cover the surface of the metal and then to add from time to time with a pipette a few drops of the fresh solution. All of the available K_3FeCy_6 is soon transformed and the reaction ceases until renewed by adding more solution.

It is sometimes an advantage for the study of single filaments or other structures to limit the number formed from the metal.

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[1] R. S. Lillie, BIOL. BULL., 1917, Vol. 33, p. 135.

[2] *Cf.* Lillie, *loc. cit.*, pp. 157–172.

This may be done by coating the metal with paraffin and then, before placing into the solution, removing several very small areas. Only one or two structures may be formed from such exposed surfaces.

Experiments have been performed with the following metals: Fe, Zn, Cd, Co, Ni, Cu, Pb, Sn, Cr and Al. For each metal which forms a precipitate with K_3FeCy_6 there is a definite and characteristic type of precipitation structure.

The presence or absence of a protective colloid has a marked influence on the kind of structure formed. Definite tubular structures or filaments are formed from Zn, Cd and Co only in the presence of a protective colloid; in its absence most of the precipitate is deposited in an "amorphous" state. Copper differs from these metals in forming filaments readily in the absence of the protective colloid.[1] It forms its characteristic structures in a 4 per cent. solution of K_3FeCy_6 in distilled water containing 4 per cent. NaCl.

III. *Character of Structures Produced with Different Metals.*

1. *Iron.*—Iron gives rise to a greater variety of well defined precipitation-structures than any of the other metals used. The structure most frequently formed is a hollow filament circular in cross-section and somewhat tapering to the end. Such a filament has a typically straight or slightly curved form if the latter is not influenced by some modifying external condition. The smaller filaments are very easily influenced and are therefore less regular in the direction of growth than the larger filaments. Figs. 3, 4 and 5 show some of the typical iron ferricyanide structures. All were photographed from the same preparation at intervals of 10 minutes, 1 hour, and 24 hours, respectively, after placing in the salt solution (2 per cent. egg albumin solution containing 2 per cent. K_3FeCy_6 and 0.5 NaCl).

In the earlier stages of development these structures are thin walled, translucent, and comparatively smooth. Later the walls become thicker and more opaque. The filaments shown in Fig. 3 are all under the surface of the solution and the presence of a distinct double contour proves beyond doubt that even the very smallest of these structures are tubular. The walls of these filaments are precipitation-membranes; in the interior of the filament is a dissolved salt of the metal, which upon coming in contact with the ferricyanide solution outside forms the precipitate upon which the growth of the structure depends. A detailed description of this process as well as a very probable explanation of the cause of the outflow of the solution has been given by R. Lillie.[1]

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[1] Lillie, *loc. cit.*, p. 141.

The phenomenon of branching, although infrequent in iron filaments, occurs under certain conditions (Fig. 6). No branching has·been observed except with the larger filaments and as a rule only with those which run along the surface of the solution. Some few cases, however, have been observed where the entire structure was submerged during its formation.

Slowness of growth is an essential condition for branching. Large filaments like those shown in Fig. 6 grow at the rate of 30 to 60 microns per minute, while the small filaments shown in the same photograph grow at the rate of 120 to 300 microns per minute. It required about 2 hours for the large branching structure shown in Fig. 6 to form. It is approximately 3 mm. long. The branches are always formed within a short distance of the growing end of the filament. It is probable that they are given off at thin or weak places in the walls where rupture and outflow of solution occur.

In general it has been found that the slower the rate of growth—that is, the nearer it approaches that of organic growth—the more resemblance there is to an organic structure. There is no doubt that the rate of the local chemical reaction, *i. e.*, the rate of formation of the precipitate, is an essential factor in determining the final form of the structure. In every case where there was a decided change in the rate of growth, there was a modification in form. The subject of the relation of rates of growth to form has been studied extensively in living organisms. D'Arcy Thompson,[2] in his book "Growth and Form," maintains that the final form which an organism assumes is entirely dependent on rates of growth. He says, "the phenomenon of rate of growth deserves to be studied as a necessary preliminary to the theoretical study of form, and, mathematically speaking, organic form itself appears to us as a function of time."[1] The rate of formation of all these structures, which depends on the rate of local electrolysis, is always most rapid at first and slows down as the reaction continues. Both internal and external conditions enter to cause the retardation. The frictional resistance to the outflow of the solution from the tubular filament and the electrical resistance of the circuit are proportional to the length of the filament. As these increase the rate at which the precipitate is formed decreases; the rate of growth is consequently lowered. There is an analogy here to the formation of living organic structures, where growth is always more vigorous at first and slows down as development continues. Child says, in his chapter on rate of metabolism, "everywhere the rate of growth is high in the young organism or in the young cells and tissues of the organism, and decreases as development proceeds and the rate of metabolism falls. With adequate nutrition and external conditions which permit growth the rate of growth appears to be in a general way dependent on the rate of metabolism";[2] and he points out that the accumulation of structure formed in metabolism may itself impede the metabolic process by which more structure is formed. The case of precipitation-structures offers an interesting analogy.

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[1] Lillie, *loc. cit.*, pp. 144–146.

[2] D'Arcy Thompson, "Growth and Form," University Press, Cambridge, 1917.

The influence of external conditions on rate and character of growth will be discussed below.

Branching can be produced in large filaments by perforating their walls with a finely pointed needle or glass rod. If this is done while the filament is growing, the branches resemble the main filament in almost every particular (Fig. 7). As many as seven branches were produced in this way, each new branch being formed from the preceding one, and sometimes three were growing at the same time. Puncturing the membrane after a filament has ceased to grow forms small side branches which themselves almost immediately cease to grow (Fig. 8).

Cross-striation is frequent in Fe filaments as well as in filaments formed from other metals. This phenomenon occurs very regularly in the larger filaments that run along the surface of the solution, and occasionally in the smaller ones. The striations in the large filaments are usually about 25 microns apart (Fig. 9). No observation has been made on the precise mode of formation of striation in iron ferricyanide structures. Usually striation indicates periodic formation; and in the case of the similar striations in Cd or Zn precipitation-structures a direct connection with periodic precipitation can be observed. Probably the conditions determining the striation in iron filaments are essentially similar, although differing in detail.

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[1] Thompson, *loc. cit.*, p. 51.

[2] C. M. Child, "Senescence and Rejuvenescence," Chapter XI., the Rate of Growth, p. 276.

Mechanical obstacles placed in the path of growing filaments have no appreciable influence on the regularity of striation. Fine grains of sand were sprinkled into the salt solution and the filaments allowed to grow out among them. The effects are shown in Fig. 10. Although the filaments were broken up and caused to branch, in some cases entirely enclosing some of the sand particles, striation continued as before. Fig. 11 is a photograph of filaments in which the direction of growth was changed by placing a few drops of water from a pipette in the solution just beyond the growing end of the filaments. A thin precipitation-membrane was formed over the end of the filaments and growth ceased for a moment. Soon the solution inside forced its way through the thin membrane and new filaments were formed which grew back into the more concentrated solution. The enlarged regions of these new filaments represent osmotic effects. It will be observed that striations are formed, but that the distances between the striæ are more variable (25 to 50 microns).

2. *Zinc.*—When a small strip of zinc, in contact with a nobler metal (Cu), is placed in a 2 per cent. egg albumin solution containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl the entire surface becomes covered within a few minutes with a dense network of filaments (Fig. 12). These structures are tubular and show some cross-striation, but differ in almost every other respect from the iron filaments just described. In minute structure they are coarsely granular with a less sharply defined outline, the double contour not showing except in a few of the

filaments which run along the surface of the solution. The manner of formation of zinc filaments, described in detail below, presents one of the most interesting rhythmical phenomena seen among any of the precipitation-structures. The rhythmic motion at the end of the filament is no doubt the cause of the regularly striated surfaces. These effects can scarcely be seen in a microphotograph on account of the coarse, thick walls of the filaments. In Fig. 13 some of the larger zinc filaments are shown. Some of these are very regularly and evenly striated. The enlarged place in one of the filaments is an instance of a broadening and flattening effect produced when a filament comes to the surface of the solution. Filaments in all cases are circular in cross section while entirely submerged in the solution.

Branching is a common feature of these large slowly growing zinc filaments. The entire outline of these structures is very irregular (Fig. 14); apparently there is a tendency to give off branches all along the course of growth.

Fig. 15 shows a part of an extremely large zinc ferricyanide filament which gave off many lateral branches. It reached a length of about 2.5 centimeters. These very large filaments grow so slowly that the deposit of precipitate can scarcely be seen under the low power of the microscope. The example shown was photographed after 24 hours in the solution. Branching can also be produced in these large growing filaments by puncturing with a fine pointed needle.

3. *Cobalt.*—In minute structure the cobalt ferricyanide precipitation-filaments resemble those formed from zinc. They develop best in a 2 per cent. egg-albumin solution containing 4 per cent. K_3FeCy_6 and 1 per cent. NaCl, and when the cobalt is in contact with copper or some other nobler metal. They resemble zinc filaments in the coarseness of the precipitate, which imparts a granular appearance and irregular outline to the filaments. There is also much non-coherent precipitate scattered along the sides of the filaments. Fig. 16 shows the characteristic structures. The manner of formation of the filaments seems to be the same as for iron filaments,—an even outflow of solution and regular deposit of precipitate. When the Co-Cu combination is put into a weaker solution (2 per cent. egg albu-

men containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl), numerous hollow, vesicular, shell-like structures (Fig. 17) are formed in addition to the slender tortuous filaments. These precipitation-vesicles closely resemble those formed on cadmium. They are finely and evenly striated, the striæ being only about 5 microns apart. They grow so slowly that no rhythmic motion can be detected, but it is probable that their formation is periodic, as in the case of the cadmium precipitation-vesicles.

A highly characteristic feature of the cobalt filaments is their tortuous, tendril-like form. They curl and wind in all directions without any apparent cause. Nickel filaments show a similar behavior. The nature of the precipitate formed, no doubt, has much to do with this phenomenon. The long filaments show no striation and there is no tendency to form branches.

4. *Cadmium.*—The most characteristic structures formed from this metal are hollow shell-like growths (Fig. 18). These form best in a 2 per cent. egg albumen solution containing 2 per cent. K_3FeCy_6 *plus* 0.5 per cent. NaCl. The structures formed under these conditions are striated and exhibit a very regular rhythmic motion (see below, page 259). They are of various shapes and sizes; occasionally they resemble a mussel-shell both in shape and character of striation. The striations vary in width in different cases from 20 to 30 microns. In Fig. 19 some of the same structures are shown after 24 hours in the solution. The largest of these are about 1 mm. in length. As is shown, their surface becomes entirely covered with fine short filaments giving them somewhat of the appearance of a burr. Very little growth takes place in the main structure after these fine filaments begin to form. As the reaction continues the enclosing membrane becomes thick and firm and more resistant to the forces which cause the rhythmic motion and periodic out-pushing of the structure. The small, short filaments then shoot out quickly at very regular intervals at different points over the surface. Some filaments are also formed directly from the surface of the metal. These are coarse and granular in appearance and show much non-coherent precipitate scattered along their course. If a strip of cadmium is put in contact with a nobler metal, or if a more concentrated solution of ferricyanide

is used, more filaments and fewer shell-like structures are formed, but in general cadmium shows little tendency to form filaments.

5. *Copper.*—Precipitation-structures are not formed so readily from copper as from Fe, Zn, and Cd and a few other metals. The solution-tension of copper is low, hence the metal does not readily enter into solution under the conditions of these experiments. A fine copper wire in contact with a nobler metal (Pt) or carbon, and immersed in a solution of 4 per cent. K_3FeCy_6 in distilled water containing not less than 4 per cent. NaCl, will in a short time form some filaments and numerous structures having the appearance of a burr (Fig. 20). The filaments that are formed are usually not large, are comparatively straight, and their walls are smooth and finely granular; very little non-coherent precipitate is formed. A few which run over the surface of the solution show a very perfect cross striation. None of these filaments show any tendency to branch. The burr-like structures are vesicular with short spicular filaments radiating out in all directions. They often exhibit a pulsating or rhythmic motion, one or more of the spicules shooting out rather quickly at each pulsation. On account of the darkness of color and thickness of the walls of these structures no striations have been detected, and few details can be shown in a photograph.

6. *Nickel.*—Strips of nickel in contact with copper or silver in a 2 per cent. egg albumen solution containing 4 per cent. K_3FeCy_6 and 4 per cent. NaCl soon form a great many winding tortuous filaments, closely resembling those formed from cobalt (Fig. 21). Some large, thick walled, irregular filaments also grow out very slowly from the surface of the metal, and large masses of amorphous precipitate are formed. Some of the filaments on the surface of the solution exhibit a few irregular cross striations. A few groups of small vesicular structures also form from the surface of nickel; these have a rhythmic motion during their formation and will be described later.

Experiments similar to the above were performed with Cr, Pb, Sn, Mn, Ag and Al, but no well defined precipitation-structures were formed except in the case of Cr and Pb. These two produced only a few very small filaments—so small in fact that they could not easily be studied under the high power of the microscope. Masses of amorphous precipitate were formed on Mn and Al, but Ag never showed any reaction, although left in the ferricyanide solution for hours at a time.

IV. *Conditions Modifying Form and Rate of Growths.*

1. *Electric Current.*—(*a*) The suggestion had been made that rate of growth of filaments could be accelerated or inhibited by electric influence.[1] Iron wires were made the electrodes in a ferricyanide solution by connecting to a battery through a rheocord, pole-changer and key. The strength of current required to affect the rate of growth is very small. By use of the rheocord the potential of the current could be altered at will. The general results were that both the rate of growth and the number of structures formed were accelerated or increased at the anode (Fig. 22) and retarded or decreased at the cathode (Fig. 23). By use of the pole-changer the current could be sent through the solution in the opposite direction, and the promoting and inhibiting effects would then show on the opposite wires. This experiment indicates clearly the electrical nature of the conditions controlling precipitation-formation from metals.

(*b*) A galvanic current from a storage battery was sent through a fine iron wire immersed in the solution. The result was both to increase the rate of growth and to change the shape of the structure. Upon making the current the rate of growth is increased and the growing filaments bulge to almost double their original diameter. By alternately making and breaking the current the filaments could be made to resemble a string of beads (Figs. 24 and 25). These effects are most probably due chiefly to the heating eflects of the current.

2. *Sudden Changes of Concentration of the Solution.*—By removing the solution from around a filament-forming metal and then immediately replacing it with water the growing ends of filaments are caused to swell out in a manner shown in Fig. 26. After such an experiment growth usually ceases, and the walls of the filaments soon break down and fall apart in a peculiar manner as shown in Fig. 27. These are osmotic effects, and show that the walls of these structures are semipermeable.

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[1] Lillie, *loc. cit.*, p. 157.

3. *Condition of Surface of the Metal.*—Oxide-covered areas on the surface of a metal form local cathodes in the ferricyanide solution; hence filaments are formed only where the pure metal is exposed. Fig. 28 shows a piece of rusty iron wire with the rust removed along one side; filaments were formed almost exclusively on the bright surface, which is anodic. Fig. 29 shows both ends of a fine iron wire (bent into a U-shape) 1.8 mm. apart, one end being rusty the other polished bright. Filaments were formed only on the bright end. It is interesting to note that nearly all the filaments formed on the bright end were directed toward the rusty end. Apparently the main direction of growth is determined by the direction of the current-lines between anodic and cathodic areas.

4. *External Conditions.*—All filaments are extremely senstive to outside influences. Slightly jarring or causing currents in the solution sometimes changes both the direction of growth and shape of filaments. Encountering small obstacles or other filaments has the same effect. Figs. 10 and 30 show the effect of filaments encountering small particles of sand.

5. *Modifications in Filaments at the Surface of the Solution.*—Some of these effects have already been mentioned. A widening and flattening effect is common with all filaments on reaching the surface of the solution. In some cases there is a display of different combinations of colors, and very beautiful figures of various shapes are formed. Filaments formed from nickel show no interesting features while entirely submerged in the solution, but when they run over the surface they become brightly colored, showing alternate light blue and brown cross-striations with a blue border along each side. Some interesting figures having the general shape of a bunch of leaves tinted with brown form at intervals along the filaments.

Another interesting surface-phenomenon is the formation of groups of small sac-like structures. This effect is very common with zinc filaments. As soon as a zinc filament in its tortuous course comes to the surface of the solution these small structures begin to form (Fig. 12). They push out around a common center, one forming every one or two seconds, at a rate varying somewhat in different cases. At the central point there is a regular rhythmic motion which lasts for several minutes. A thin and almost transparent membrane swells up rather quickly and then a small saccule shoots out at the side; at this instant the membrane falls back, seemingly the pressure against it being relieved. This process continues for several minutes in each case and finally slows down and stops. The thin membrane never seems to rupture except at its edge where the little sac-like structures are formed. In some cases small filaments form also and radiate out around the membrane.

After several hours many such groups have formed and the whole presents a striking appearance. This phenomenon is not frequent with iron filaments, although some cases have been observed (Fig. 6). In these the vesicles are usually smaller, they form more quickly and a larger proportion of fine filaments are formed. Such structures are frequently formed from cobalt filaments and in this case are always much larger than those formed from iron or zinc (Figs. 16, 17). They vary from 200 to 300 microns in diameter, the enclosing membranes are thicker, and the whole structure is somewhat oblong in shape. The exact manner of formation has not been observed; no rhythmic motion has been seen.

6. *Influence of Mechanical Contact.*—Contact with solid objects has a marked influence on both the direction and rate of growth. In some cases filaments have shown tendril-like tendencies. Cases were observed in which Fe-ferricyanide filaments have wound spirally around a fine iron wire as often as nine times. Filaments of all the metals used show some reaction to contact with other bodies. Fig. 9 shows three filaments following along side by side for some distance.

7. The retarding and accelerating effects on the growth of precipitation structures observed in combinations of metals of different solution-tension have already been studied and described in full by R. Lillie.[1]

V. *Rhythmical Phenomena in Growing Filaments and Other Structures.*—Rhythmic motion is not evident in the formation of filaments in all cases, as, *e. g.*, in the filaments formed from iron. These seem to grow by an even and regular deposit of precipitate, although, under certain conditions the surface filaments show a regular cross striation, a feature probably indicating periodic precipitation.

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[1] Lillie, *loc. cit.*, pp. 144–146.

Under some conditions not yet clearly defined small burr-like structures are formed on the surface of an iron wire. Their formation seems to depend more on the condition of the surface of the metal than on the concentration of the solution. Some of these structures are shown in Fig. 2. They are always very small, and in the presence of a large number of filaments may easily be overlooked. During their formation they show a regular rhythmic motion causing the fine filaments on their surface to wave to and fro with a cilium-like movement. In some cases the whole structure pulsates, but in others only the filaments show any motion. This movement is comparatively slow, the rhythm being at first only about once every second, and later slowing down to once every five to eight seconds; sometimes this rhythmical motion continues for ten minutes. In the formation of zinc ferricyanide filaments very interesting rhythmical phenomena are exhibited. A small strip of zinc, in contact with a nobler metal (Cu, Fe), when placed in a ferricyanide solution becomes entirely covered with a fungus-like coat of filaments in a few seconds. At the extremity of each filament there is visible a regular rhythmic motion or pulsation, very similar to that described above as accompanying the formation of sac-like structures from zinc filaments at the surface of the solution. An almost transparent membrane swells out from the end of the filament, remains an instant, and then falls back quickly; then again the membrane slowly pushes out and the process is repeated. There is no visible evidence that the membrane is ruptured at any stage of the motion; neither can any suspended particles of precipitate be seen to flow from the end of the filament. The filament slowly increases in length, and the rate of increase seems to be proportional to the rate of rhythmical motion. It seems probable that each time the pulsating membrane falls back, it has just been ruptured at its edges, some precipitate being then formed and deposited. The rate of the motion varies from about once per second in the larger filaments to five or six per second in the small ones. No such movement is seen in the large filaments on the surface of the solution. During the formation of some of the larger filaments having this motion, small spicule-like filaments grow out radially from the edge of the pulsating membrane. One or more forms at each pulsation until, when the filament ceases to grow, the whole surface is covered with the small sharp filaments and presents a bristly appearance.

A similar rhythmic motion has been observed during the formation of groups of small structures on the surface of nickel. Here also the pulsating center is present, but small saccules, about 30 to 60 microns in diameter, are formed around the membrane instead of small filaments as in the case of zinc.

The burr-like structures formed on copper have the same rhythmic motion as the small structures which sometimes form on iron. They exhibit both the pulsating movement of the vesicle as a whole and the cilium-like motion of the separate filaments. The rate of movement is usually about three per second.

Cadmium ferricyanide precipitation-structures have a more regular rhythmic motion than in any other case so far observed. The structures are large enough to enable the exact manner of formation to be seen. Each structure originates at a minute anodic region over which a small precipitation membrane is formed, and the succeeding increase in the size of this membrane takes place periodically. Growth is not all in the same direction; hence, characteristic shell-like structures are formed instead of filaments (Fig. 18). The increase in size takes place through a periodical rupture and reformation of the enclosing membrane. At each rupture a slight outflow of solution occurs, but a new membrane is at once formed, closing the opening; in this way the structure is enlarged by the amount of new membrane formed during the pulsation. When this increase in surface takes place each time at a region whose position (relative to the metal) remains unchanged, the structure formed is regular and symmetrical in shape. Sometimes, however, the rupture is first at one region and an instant later at another; this tends to make the structure irregular and asymmetrical in shape. The surfaces of the structures thus formed are finely striated, the striæ varying in width from 20 to 30 microns. The direction and arrangement of the striations depend on the manner in which the growth has taken place. On the symmetrical structures the striations are regular and equidistant. On the whole these precipitation-formations bear a very decided resemblance to group of small shells.

Rhythmical movements similar to those observed in these precipitation-structures are met with in various other inorganic processes. Periodicity in purely chemical reactions and in the formation of osmotic growths, as well as in the growth and development of organic structures, has long been known. One of the best examples of chemical periodicity is seen in the so-called Liesegang rings,[1] which are formed by a diffusion-process associated with regular periodic precipitations. Leduc,[2] in his book "Mechanism of Life," gives a full chapter to periodicity in chemical and other phenomena. He describes certain osmotic structures as having rhythmic motion and as growing periodically.[3] In Child's book, "Senescence and Rejuvenescence," considerable space is given to the subject of periodicity in organisms.[4] In this discussion he shows that the growth of an organic structure is not an even, uninterrupted development, but is a largely periodic phenomenon. Another interesting and curious phenomenon is the periodic catalysis of hydrogen peroxide by mercury, as described by Bredig.[5] The liberation of gas in the catalytic action takes place intermittently and may continue at regular intervals for an hour or more. An electrical current of action is also produced resembling that produced in the rhythmic contraction of the heart, and can be registered in the usual manner by a string galvanometer. All of these purely chemical and physical phenomena are significant in that they point the way to a better understanding of the phenomena of rhythm and periodicity in living beings.

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[1] *Cf.* Liesegang, "Beiträge zu einer Kolloidchemie des Lebens," Dresden, 1909; Stansfield, "Retarded Diffusion and Rhythmical Precipitation," *Amer. Journ. Sci.*, 1917, Vol. 43, p. 1.

[2] Leduc *loc. cit.*, pp. 67–77.

[3] Leduc, *loc. cit.*, p. 156.

[4] Child, *loc. cit.*, pp. 187–193.

[5] *Cf.* Bredig and Wilke, "Erregung und Beeinflussung katalytischer Pulsationen durch elektrische Ströme," *Biochem. Zeitscher.*, 1908, Vol. 11, p. 67.

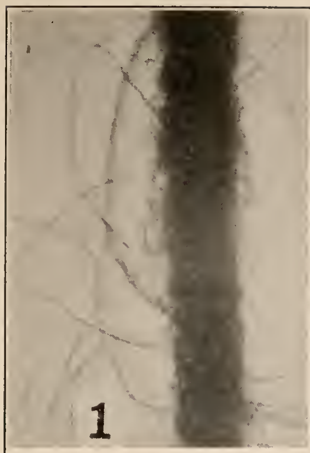
DESCRIPTION OF PLATE I.

Unless otherwise indicated the solution used for the production of the different structures was a 2 per cent. egg albumen solution containing 2 per cent. K_3FeCy_6 and 0.5 per cent. NaCl. In all these photographs the magnification is 32 diameters. In most cases the structures were allowed to develop while in position for taking the photograph, in order to avoid modifications produced by moving the preparation.

FIG. 1. Fe-ferricyanide filaments, 5 min., after placing in solution (2 per cent. egg-albumin, + 4 per cent. K_3FeCy_6 + 1 per cent. NaCl).

FIG. 2. Fe-ferricyanide structures, about 10 min., after growth began.

FIGS. 3, 4 AND 5. Fe-ferricyanide filaments photographed after 10 min., 1 hr., and 24 hrs., respectively. From the same preparation.



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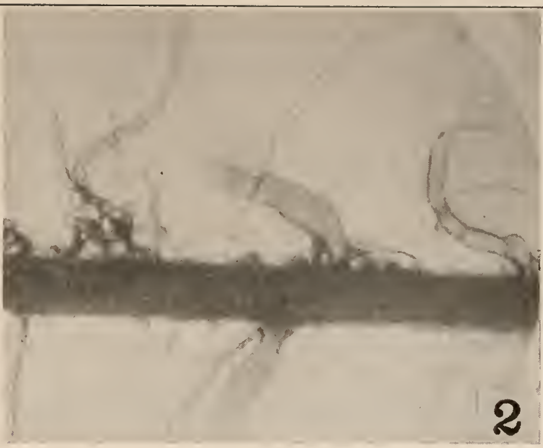

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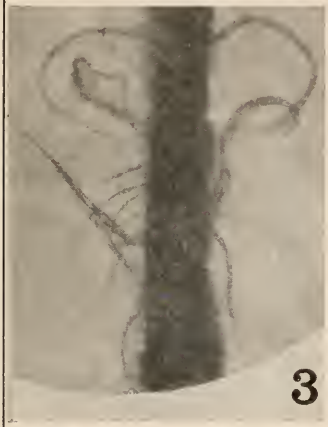

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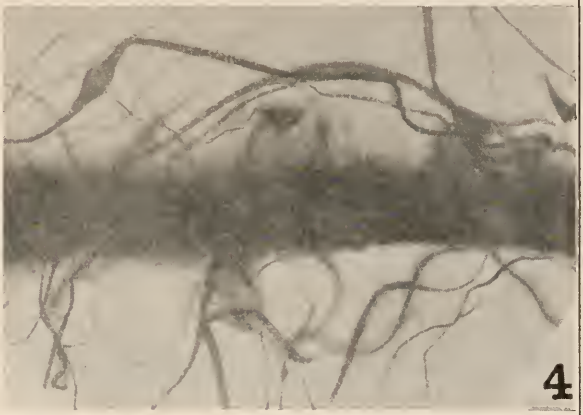

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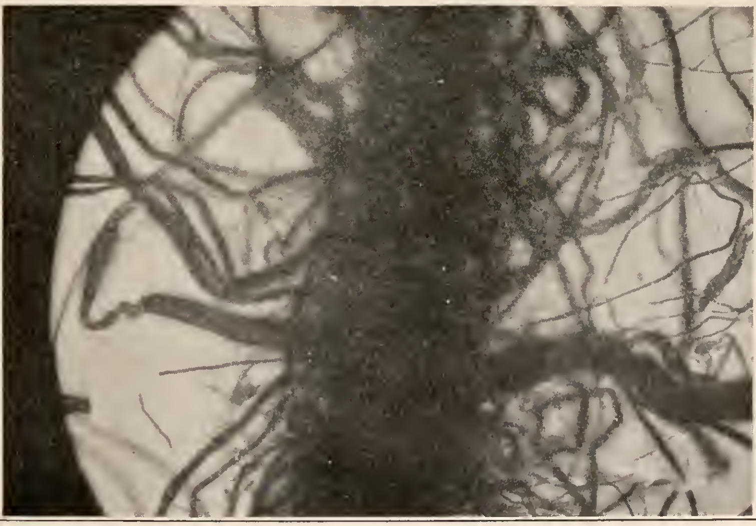

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Description of Plate II.

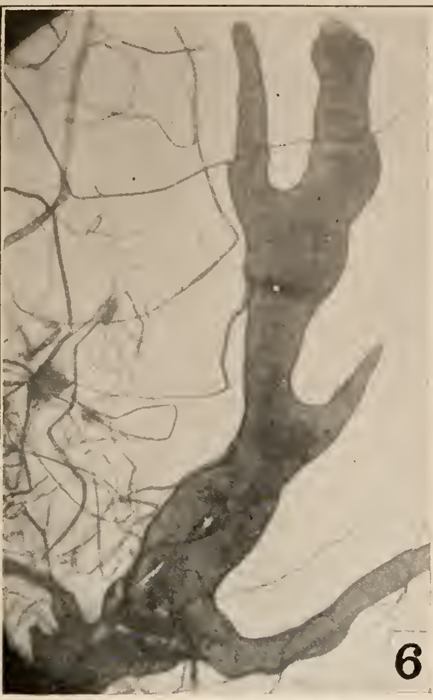
Fig. 6. Fe-ferricyanide structures, on the surface of the solution. Striæ about 50 microns apart.

Fig. 7. Branching produced in a growing Fe-ferricyanide filament by puncturing with a finely pointed needle. In the corner is a branch formed naturally.

Fig. 8. Branches produced by puncture in an Fe-ferricyanide filament after growth had ceased.

Fig. 9. Fe-ferricyanide filaments finely striated. Striæ about 25 microns apart. These filaments are on the surface of the solution.

Fig. 10. Large Fe-ferricyanide filaments, on the surface of the solution, broken up by fine particles of sand.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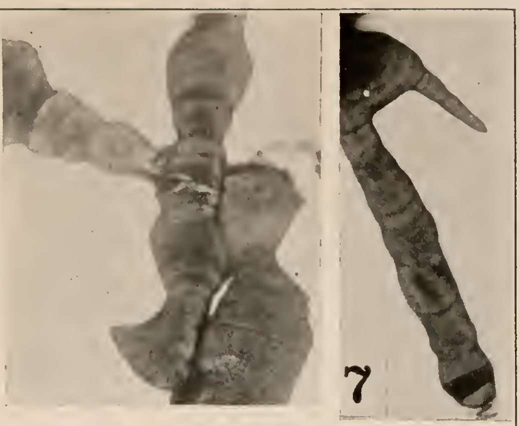
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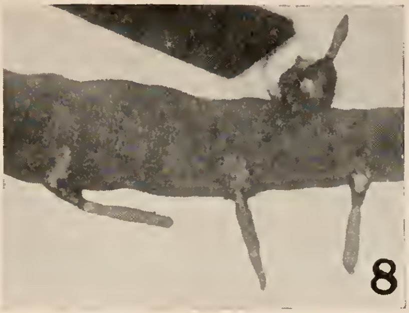
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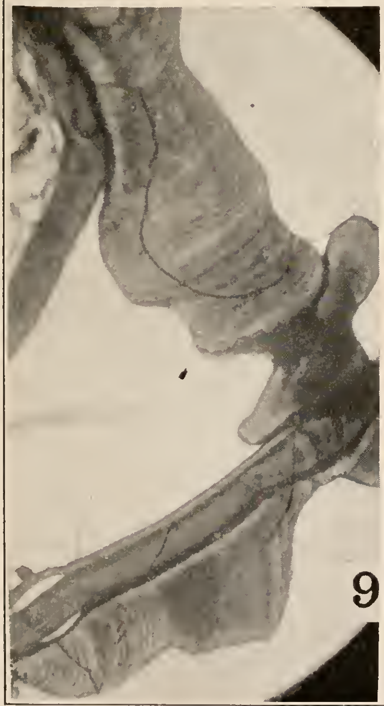
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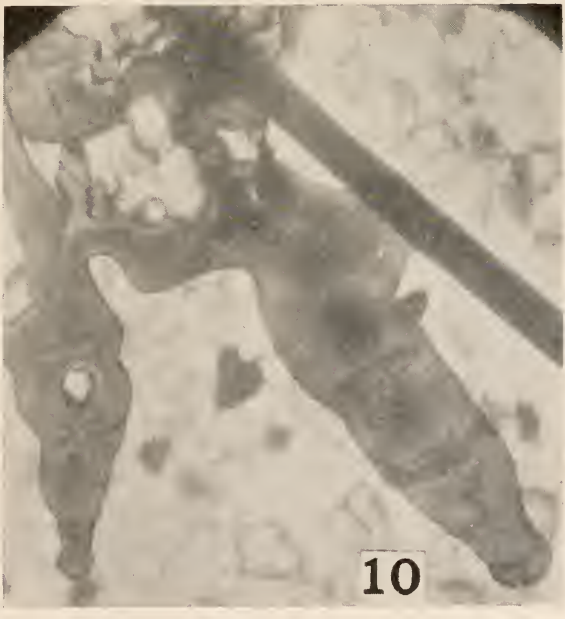
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Description of Plate III.

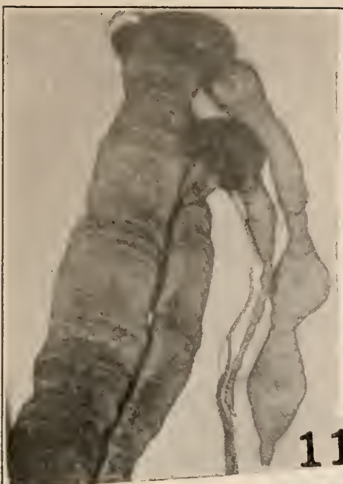
Fig. 11. Large Fe-ferricyanide filaments showing the effect of dropping water in the solution just beyond the growing end of the filaments. The striæ were 25 to 50 microns apart before the change of concentration.

Fig. 12. Zn-ferricyanide filaments, and saccules formed at the surface of the solution.

Fig. 13. Large striated Zn-ferricyanide structures. The copper wire is shown wound about the Zn-strip.

Fig. 14. Zn-ferricyanide filament showing the irregularity of growth at the surface of the solution and the general tendency to branch.

Fig. 15. Part of a large, branching Zn-ferricyanide filament on the surface of the solution. Photograph after 24 hrs., in the solution.



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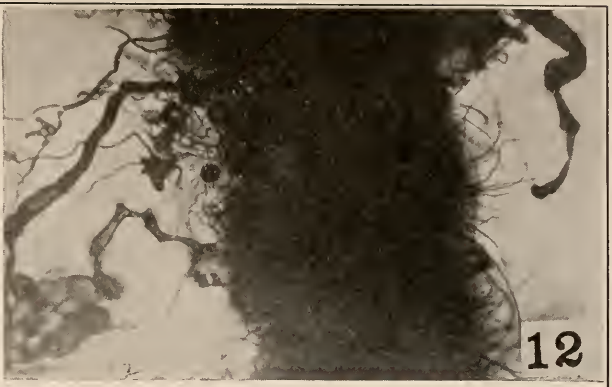

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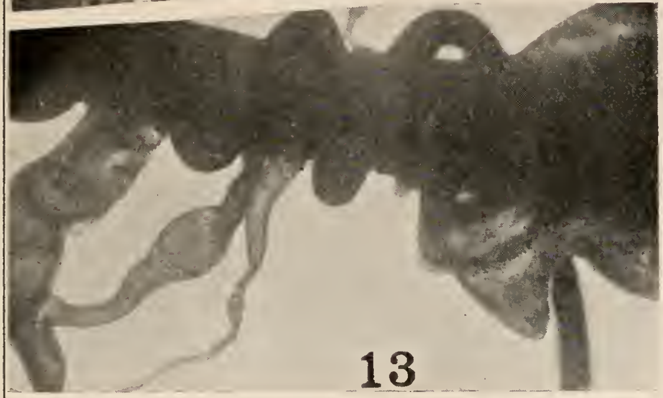

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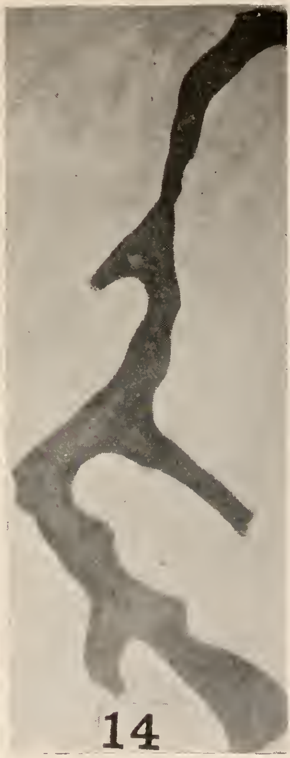

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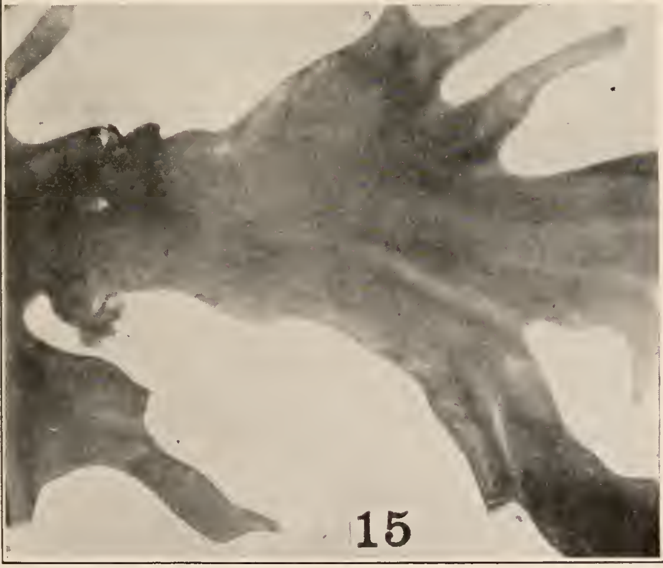

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DDSCRIPTION OF PLATE IV.

FIG. 16. Co-ferricyanide structures formed in 2 per cent. egg albumin + 4 per cent. K_3FeCy_6 + 1 per cent. NaCl. A group of surface saccules shows at one side.

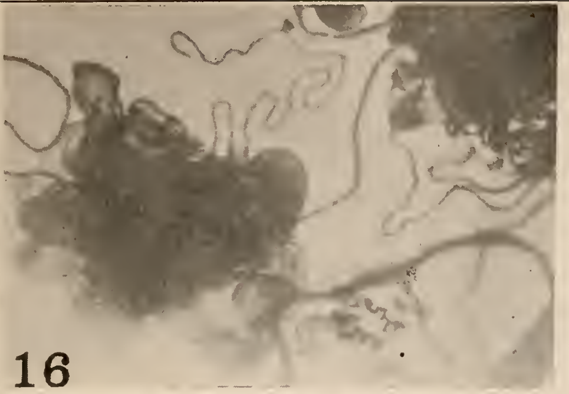
FIG. 17. Co-ferricyanide filaments and striated shell-like structures at the surface of the metal. The tortuous filaments are submerged in the solution.

FIG. 18. Cd-ferricyanide structures. The longest of these structures are about 320 microns. It required about 2 hrs., for these to form.

FIG. 19. Cd-ferricyanide structures photographed after 24 hours in the solution The largest are 1.1 mm. long.

FIG. 20. Cu-ferricyanide structures. Formed in a 4 per cent. solution of K_3FeCy_6 in distilled water + 4 per cent. NaCl.

FIG. 21. Ni-ferricyanide precipitation filaments. Formed in a 2 per cent. egg albumin solution + 4 per cent. K_3FeCy_6 + 1 per cent. NaCl.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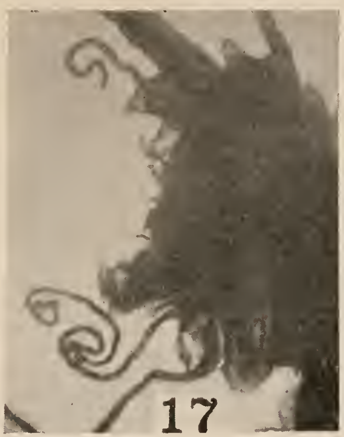
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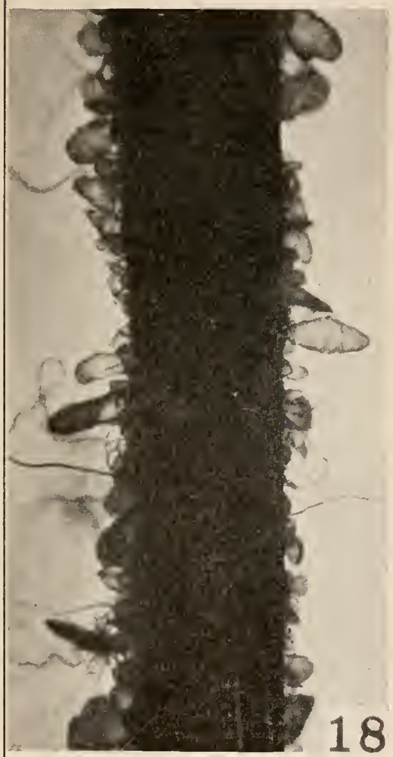
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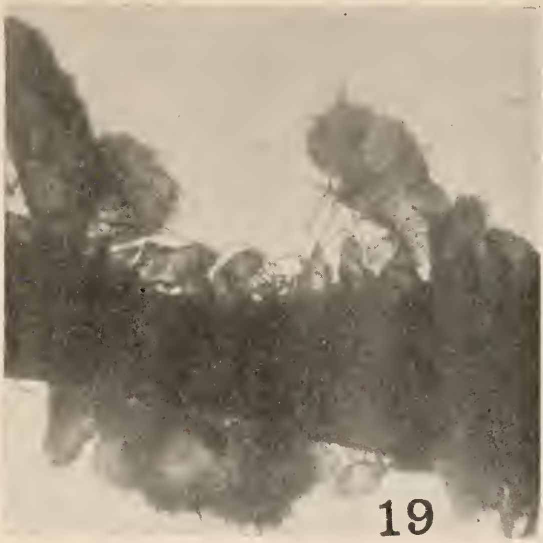
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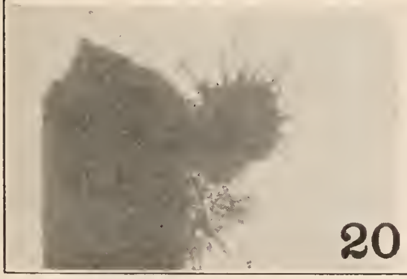
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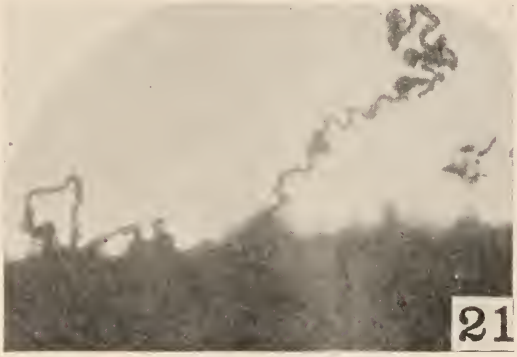
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Description of Plate V.

Fig. 22. Fe-ferricyanide growths, showing the acceleration caused by making metal anodic while the structures are forming.

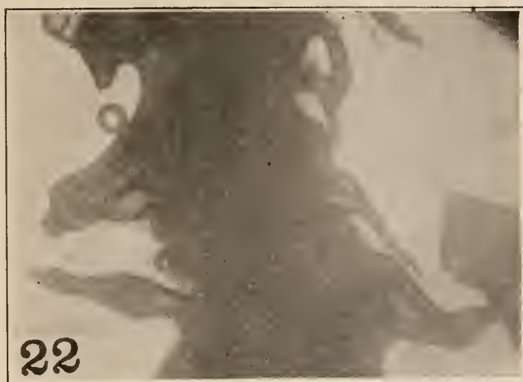
Fig. 23. An iron wire in a ferricyanide solution showing cathodic inhibition of precipitation formations. The round objects are hydrogen bubbles.

Fig. 24. Filaments showing modifications produced by passing a galvanic current directly through the metal while the filaments are forming. The beaded effect is produced by alternately making and breaking the current.

Fig. 25. Same as Fig. 24.

Fig. 26. Precipitation filaments from iron, showing enlargements at the ends of the filaments produced by a sudden decrease in the concentration of the solution·

Fig. 27. This shows the peculiar manner in which large Fe-ferricyanide filaments break down after a sudden lowering of the concentration of the surrounding solution.



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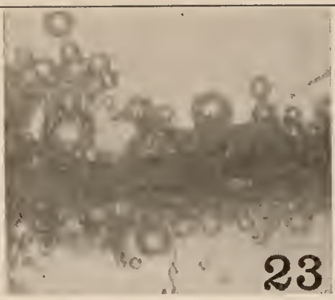

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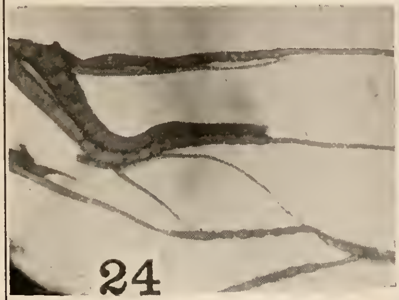

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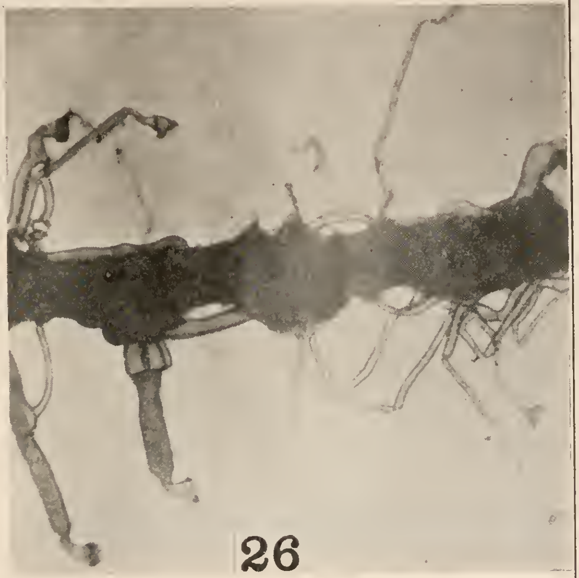

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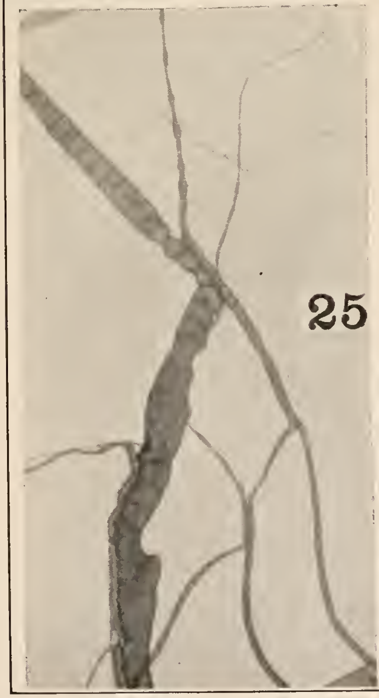

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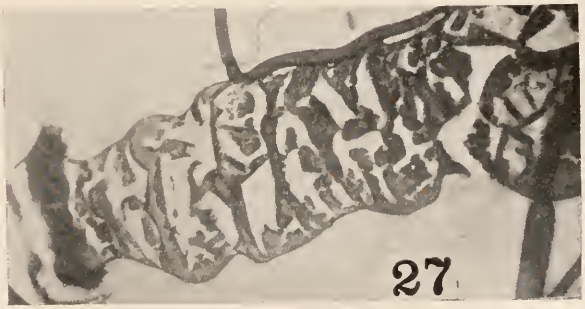

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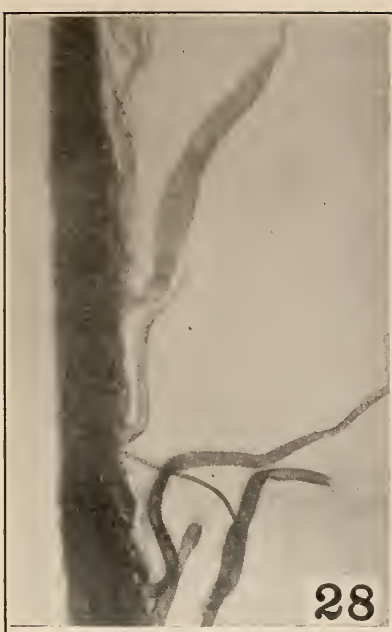
RALPH S. LILLIE AND EARL N. JOHNSTON.

Description of Plate VI.

Fig. 28. A rusty iron wire forming filaments only on the bright surface from which the rust had been removed. All rust-covered surfaces are cathodic.

Fig. 29. Both ends of a U-shaped iron wire in a ferricyanide solution. One end is rusted and forms no filaments, the other is polished bright, hence is anodic and forms many filaments.

Fig. 30. This shows the modification in direction of growth and shape of filaments caused by small obstacles (fine sand) in the solution.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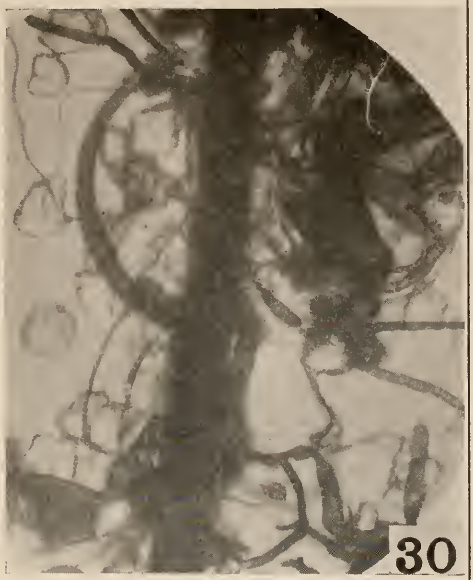
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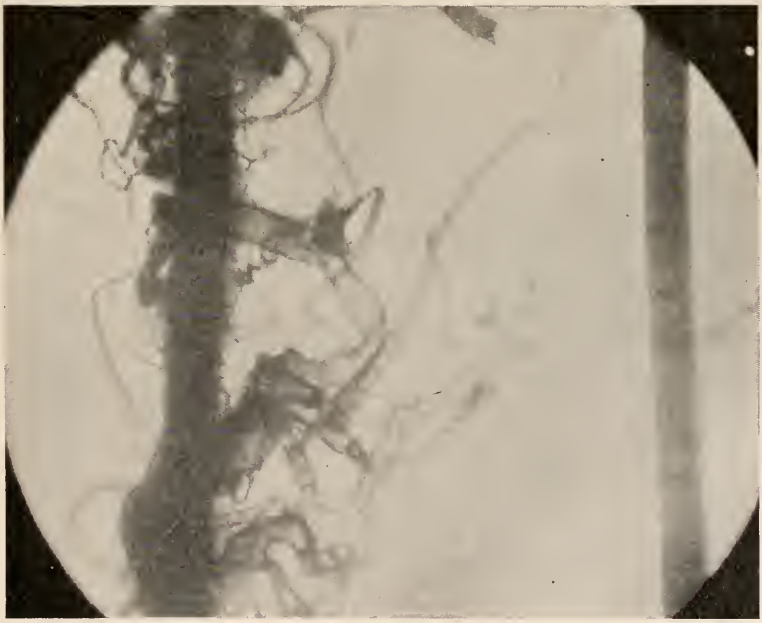
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RALPH S. LILLIE AND EARL N. JOHNSTON.

TWO CILIATA OF GREAT SALT LAKE.[1]

DEAN A. PACK.

INTRODUCTION.

Great Salt Lake is not a body of water without its natural fauna and flora. Its water has not yet reached the point of saturation for sodium chloride. It is not the lake of no life, whose water is thought, by the public in general, to be purified by the abundance of salt in solution. It is a lake that contains probably less than fifty forms of life, some of which are visible to the naked eye. Upon careful microscopic examination of a generous amount of the Great Salt Lake water (23 per cent. salinity) many forms of life are observed. To date there have been seventeen different forms reported; as nine algæ, five bacteria, two protozoans, one crustacean and two fly larvæ.

The plan of work was to study the effect of dilution on Great Salt Lake forms. Out of a number of algæ and protozoans, two ciliata were chosen for experimentation.

MATERIAL.

The material was collected early in September, 1917, from near the shore of Great Salt Lake in the vicinity of the Salt Air Pavilion and permitted to stand in the laboratory until January, 1918. As no water was added to the material, it concentrated to a density of 1.170 at 60 degrees Fahrenheit, by the first of January, 1918, when the first dilutions were started. A good balance between the animal and plant life was established and the cultures retained their clearness through all the different densities from the point of saturation down to the density 1.015 (at date).[2] The decaying and milky cultures spoken of by other observers were not encountered. The *Aphanothece* and some other plants grew and reproduced, in all the different densities from 1.20 to 1.010, thus serving as animal food and giving the cultures their characteristic lake green color.

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[1] Contribution from the Zoölogy Department of the University of Utah.

[2] August 1, 1918.

The number of living organisms appearing was so large in the first cultures, that contamination was suspected. Consequently collections for checking were made April 30, 1918, under all the precautions that one takes in the collection of water samples for bacteriological examination. The material was examined and found to contain the two ciliata described later in this article and some other forms in common with the September material.

Dilution was effected gradually and accurately, by a method of continuous diluting. The process consisted in adding enough distilled water to a culture to lower its density .0025 or one fourth of one per cent. every 24 hours. This method was adopted for several reasons; first, one does not destroy delicate forms that would perish when plunged into water of only one tenth the original density; second, one has before him a continuous picture of the changing life; and third, one deals with the offspring of only a few individuals.

The moist chamber made by the use of the hollow-ground slide was found to be an advantage in the study of these forms. It was made by placing a small quantity of culture, that showed an equal balance between animal and plant life, on a clean cover slip; inverting this over a deep hollow-ground slide, and sealing with vaseline. If small cultures are desired several may be made on one cover slip by means of the platinum loop. In this case a drop of culture is placed in the hollow-ground slide to prevent evaporation. A mechanical stage greatly facilitates the study.

Cultures with a predominance of animals or plants will show the effect of different gases upon the form and habits of both animals and plants. Not only can the effect of gases and liquids on living forms be studied to good advantage, but this preparation makes possible accurate study of responses to light, darkness, heat, etc. Some of these preparations were made fifty days ago and are at date in good condition.

General Discussion.

Diluting the culture medium produced certain definite changes in these two ciliata. These protozoans were found to vary with a change in the supply of food, oxygen, carbon dioxide, and light;

and also with respect to division, conjugation, and encystment. Dilution of the culture medium was accompanied by increased size, increased activity, shortening of the feeling cirri, more active physiological and reproductive processes, and more flexible and contractile bodies.

In changing the density of the culture from 1.110 to 1.040 the Uroleptus packii increased in length from .07 mm. to .11 mm. and the *Prorodon utahensis* increased in length from .06 mm. to .08 mm. The *Uroleptus packii* cysts increased in diameter from .025 mm. to .03 mm., and the *Prorodon utchensis* cysts increased in diameter from .023 mm. to .027 mm. Like changes were also noted among other protozoans.

The protozoans showed an increased activity as the medium became less dense. All estimates of speed were made by means of stopwatch, camera, lucida, and a dial made of concentric rings reading to .01 mm. and .05 mm. The rate given for each protozoan is the average of ten records for each of ten individuals. These rates are given in the following table.

Protozoan.	Date.	Density of Medium.	Distance.	Time.	Kind of Motion.
Uroleptus packii.......	May 20	1.2	100 microns.	10 min.	steady.
“	“ 25	1.11	“ “	7.5 sec.	“
“	“ 25	“	“ “	5.6 “	darting.
“	“ 25	1.06	“ “	3 “	steady.
“	“ 29	1.04	“ “	2 ‘	steady.
“	“ “	1.06	“ “	1–2 “	darting.
Prorodon Utahensis.....	Apr. 29	1.2	100 microns.	20 min.	steady.
“	May 25	1.075	“ “	3.5 sec.	“
“	“ 25	1.045	“ “	3 “	“
“	“ 25	1.039	“ “	2 “	“

This increased activity was also very interestingly demonstrated by the rate of vibration of the feeling cirri on the *Uroleptus packii.* These ciliates, in the densities below 1.030, moved their feeling cirri hundreds of times faster than the same ciliates moved their feeling cirri when in the saturated densities. At a density of 1.22 one single stroke of the feeling cirrus occupied 15 seconds. One stroke of a feeling cirrus at the density 1.150 occupied 1 second. In the density 1.11 there were at least five strokes per second, while in the densities below 1.030 not even an estimate could be given. The increased activity, that came with less dense media, was evident in other general movements.

The feeling cirri on the ciliates from the saturated medium were between 17 and 20 microns long, while the feeling cirri on equally large ciliates from a medium with density 1.030 were between 10 and 11 microns long. The two inside feeling cirri of the ciliate in saturated medium were thus reduced by dilution to nearer the length of the two outside ones and the ventral creeping cirri. That is, our ciliate has practically replaced the two specialized feeling cirri by the less specialized cirri.

The physiological and reproductive processes were accomplished in less time in the less dense solutions. With increased dilution; the protozoans ingested more food, the waste vacuole discharged more often, and the contractile vacuole hurried up in a like manner. Reproduction occurred more rapidly and more often as the foregoing processes were speeded up.

The bodies of both protozoans became markedly less rigid as dilution went on. In normal lake water the bodies of both forms were not known to contract any appreciable amount. *Prorodon utahensis* could bend its body through an angle of 30°, while the *Uroleptus packii* was never found in a bent position. At the density of 1.06 the *Prorodon utahensis* was seen to bend through an angle of 180°: or until the anterior part of the body folded on the posterior part. At the density 1.03 the *Uroleptus packii* was seen to bend through an angle of 150°. Both forms in the lower densities could shorten and lengthen the body at will. The *Prorodon utahensis* seemed to have lost all rigidity of body, assuming readily a spherical or a cylindrical form.

These protozoans responded definitely toward light. This fact was demonstrated, first with active and second with encysted forms. The active forms after an exposure to darkness for 12 hours, swam 66 microns in 10 seconds; while after an exposure to darkness for only 6 hours, they swam 100 microns in 10 seconds. The same forms after being exposed to darkness for 12 hours followed by exposure to light for 4 hours swam 300 microns in 10 seconds. These figures show: first, that exposure to darkness was accompanied by decreased activity; second, the longer the exposure to darkness the greater the inactivity; and third, that light was necessary for maximum activity. The encysted forms for several hours before emerging exhibited periods of rest followed by periods of activity. This could be explained as fatigue. However it was found that they were more active during the day than during the night. It was also noted that individuals quieted down by exposure to darkness were excited to activity in a few minutes by exposure to a 40-watt light (distance 2 feet). These individuals were found to be more active as the intensity of light increased within the limits of photosynthesis. A suggested explanation of these results is found in the fact that the protozoans possess chlorophyll, probably in the form of a symbiotic alga, which gathers carbon dioxide and other wastes from the ciliate, recombines them into food, and liberates oxygen. The utilization of the synthesized food and free oxygen by the protozoan may account for its increased activity.

The geological history tells us that a few hundred thousand years ago Great Salt Lake was a body of fresh water. This suggests that our present salt water forms were in the past fresh water animals. The fact that these forms have withstood the the great change from fresh water to a solution of 23 per cent. salinity shows that they were capable of adaptation. Those forms that were unable to adapt themselves to the salt water have been weeded out and only plastic forms left. The results discussed in the foregoing part of this article show that these ciliata are plastic and capable of great adaptation. They adapted themselves to all densities from 1.22 down to 1.010 (to date), which is almost a complete reversal of a change covering thousands of years.

DESCRIPTION OF SPECIES.

Uroleptus packii Calkins[1] (see Fig. 1) is a pale green colored ciliate with dorsal and ventral surfaces, a tapering posterior end, and a blunt anterior end which bears two long and two shorter feeling cirri. This form is .07 mm. long (salinity 23 per cent.). The body is quite rigid; keeping the characteristic shape. The ectoplasm is easily distinguished and carries three marked rows of creeping cirri. These rows of cirri extend diagonally backward from the right anterior end to the left posterior end over the ventral surface. The row fartherest to the left is interrupted one-third of the way back by the peristomial region. This region is bounded on the left by the peristomial membranellas, which extends backward turning slightly to the right, passing through the mouth, and ending in the cytopharynx. The right side of this region is bounded by a large undulating membrane which extends backward and turns slightly to the left. A

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[1] Prof. Gary N. Calkins has seen this form and, provisionally, named it *Uroleptus packii*.

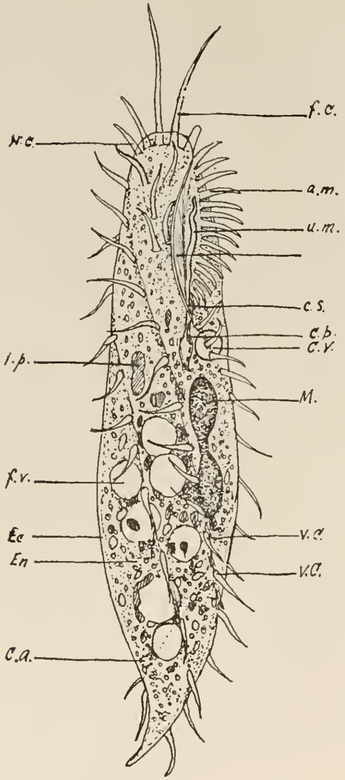

Fig. 1.

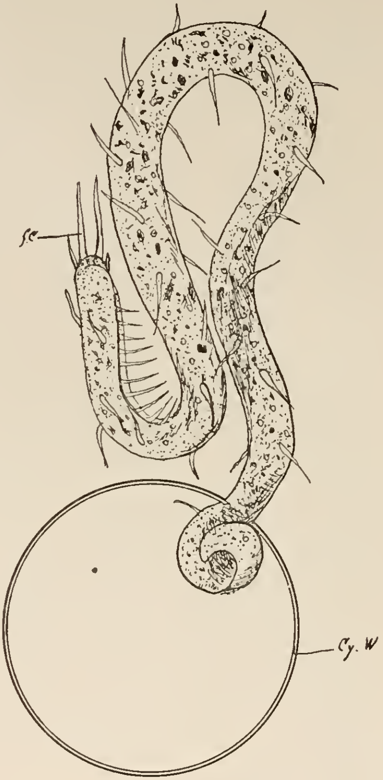

Fig. 2.

Fig. 1. *Uroleptus packii.* *fc*, feeling cirri; *frc*, frontal cirri; *am*, adoral membranellæ; *umt*, undulating membrane (tongue); *um*, undulating membrane; *cs*, cytostome; *cp*, cytopharynx; *cv*, contractile vacuole; *ip*, *Aphanothece* cell; *M*, macronucleus; *fv*, food vacuole; *Ec*, ectoplasm; *En*, endoplasm; *vc*, ventral cirri; *ca*, cytopyge.

Fig. 2. *Uroleptus packii.* *Fc*, feeling cirri; *cyw*, cyst wall.

second tongue-like undulating membrane extends out of the mouth and forward along the floor of the peristomial region. The cytopharynx is a short swollen tube that extends from the mouth backward with a left dorsal deflection. There may be as many as thirty food vacuoles in the body at one time. The remains of the food vacuoles pass out through the posterior dorsal anus. The contractile vacuole is situated in the fore part of the body. The nucleus is situated midway along the body in the left dorsal quarter. This form derives its color from chlorophyll, which is probably in the form of symbiotic alga.

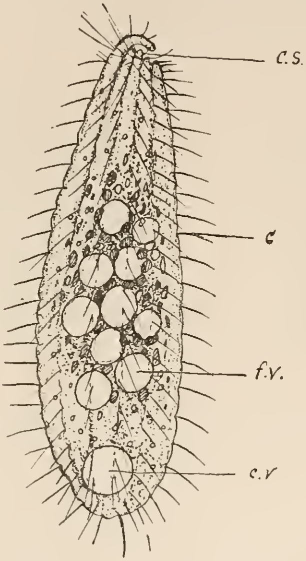

FIG. 3. *Prorodon utahensis.* *cs*, cytostome; *c*, cilium; *fv*, food vacuole; *cv*, contractile vacuole.

This form takes food in the form of bacteria, small plants and animals which abound in the water. It is a very active form, having a creeping or swimming forward movement of 100 microns per 7 seconds and a darting backward movement of double this speed. This backward movement generally follows irritation at the anterior end. This form when kept in a saturated solution was found to be sensitive to a flash of light from the mirror of the microscope.

Reproduction is brought about by binary fission. Both transverse and longitudinal methods of fission were observed. Conjugation is followed by rapid division. At certain times, perhaps after a full meal, this protozoan encysts and may remain encysted for fifty days. It finally becomes active again and breaks through the cyst wall as a much elongated form (see Fig. 2). After swimming a short time in this form it divides transversely, and if the posterior individual is not sufficiently organized it rounds up into a cyst.

Prorodon utahensis[1] (Fig. 3) is cylindrical in shape, .05 mm. long, and bears an even coat of cilia. The cilia are distributed between ridges that run parallel to the long axis of the body. These ridges project slightly beyond the opening of the mouth (anterior end) and form finger-like projections. One of which is long and well developed, being used to crowd food into the mouth. The œsophagus is short and fitted with short rods that may be extended in the shape of a star (see Fig. 4). The mouth and œsophagus are capable of being expanded to near the diameter of the body. Food vacuoles are many in number. The contractile vacuole is large and at the posterior end. The nucleus is small. The endoplasm probably contains symbiotic alga.

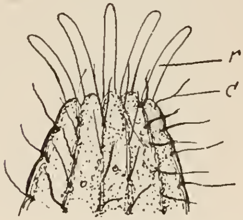

FIG. 4.

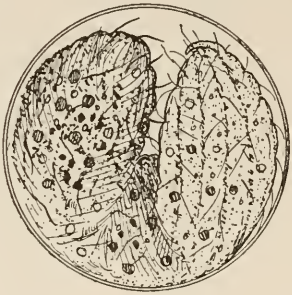

FIG. 5.

FIG. 4. *Prorodon utahensis.* Anterior end showing *r*, extended rods.
FIG. 5. *Prorodon utahensis.* Encysted.

This form is an active feeder. It lives on bacteria, plant cells, amœbæ, small ciliata, mastigamœba, and other small protozoans found in the lake. An amœba, with a diameter fully equal to that of this ciliate, lost its identity two minutes after being swallowed. At one time an amœba was swallowed that occupied fully one-half of the protozoan's body. It may feed for several hours, then quiet down, contract into a short ellipsoid during which time it extends at intervals the œsophagus rods. After about one hour of this procedure it elongates and swims away, continuing again the process of feeding.

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[1] The writer has given the name *Prorodon utahensis* to this form, which is very probably a new species.

It is an active swimmer. Reproduction is brought about in a short period, by longitudinal fission. Cysts were observed and the contained individuals were found to be sensitive to light, a fact discussed in connection with symbiotic alga.

CONCLUSIONS.

1. Though the number of animal and plant species reported from Great Salt Lake is only seventeen, evidence goes to show that this number will at least be doubled.

2. The milky cultures are the result of an unbalanced animal and plant relationship.

3. Continuous dilution was an advantage in that it did not destroy delicate forms, and the offspring of only a few individuals were studied.

4. The two ciliates responded to dilution of the medium by increased size, increased activity, shortening of the feeling cirri, more active physiological and reproductive processes, and more flexible and contractile bodies.

5. These protozoans contain chlorophyll, probably in the form of a symbiotic alga.

6. The results of the foregoing experiments show that these ciliates are plastic and capable of adaptation.

7. It is believed from the foregoing that by slowing down the rate of dilution, some of these Great Salt Lake forms may be transformed into fresh water animals.

8. Most cordial thanks are due Professor Newton Miller, who followed the work with interest and gave many helpful criticisms.

BIBLIOGRAPHY.

Fremont, John C.

'43 Report of the Exploring Expedition to the Rocky Mountains in the year 1842, and to Oregon and North California in the years 1843–4, Washington, D. C., 1845.

Verrill, A. E.

'67 Twelfth Annual Report U. S. Geological and Geographical Survey of the Territories of Wyoming and Idaho. Part 1, pp. 330, Washington, D. C., 1878.

Siebold,

'78 Twelfth Annual Report U. S. Geological and Geographical Survey of the Territories of Wyoming and Idaho. Part 1, pp. 459–463, Washington, D. C., 1878.

Packward, A. S., Jr.

'78 Same. Part 1, pp. 295–592, Washington, D. C., 1878.

Farlow.

'79 A Description of a Blue-green Alga, *Polycystis packardii*, 1879.

Jordan, David Starr.

'89 Report of Exploration of Colorado and Utah during the Summer of 1889, Bulletin of the U. S. Fish Commission, Vol. IX, pp. 31, Washington, D. C., 1889.

Gilbert, Grove Carl.

'90 Lake Bonneville, p. 259, Washington, D. C., 1890.

Rothpletz, A.

'92 Report on *Gleothece* and *Gleocystis*. Bot. Centr., p. 35, 1892.

Moore, H. F.

'99 The Feasibility of Introducing Useful Marine Animals into the Waters of Great Salt Lake. 1899.

Talmage, James E.

'00 The Great Salt Lake—Present and Past, pp. 67–76. Salt Lake City Utah, 1900.

Vorhies, C. T.

'17 Notes on the Fauna of Great Salt Lake. The American Naturalist, Vol. L., pp. 494–506.

Daines, L. L.

'17 On the Flora of Great Salt Lake, The American Naturalist, Vol. L., pp. 499–506.

Jensen, Albert C.

'18 Some Observations on *Artemia graeilis*, the Brine Shrimp of Great Salt Lake. Biol. Bull., Vol. XXXIV., No. 1, pp. 18–25.

UTERINE, TUBAL AND OVARIAN LYSIS AND RESORPTION OF CONCEPTUSES.[1]

ARTHUR WILLIAM MEYER.

It has long been known that considerable intrauterine retrogression of a conceptus can occur in multiparous mammals. D'Outrepont, for example, on page of the 192 catalog of his collection, is said to represent the uterus of a rabbit, in which an apparently full term fetus is found near the distal end of one horn, although the approximate portions of both horns contained nothing but remnants of fetuses—one in each. According to Muller, '47, the enlargement in the right horn contained a "cyst filled with rabbit hair imbedded in a soft mass and that on the left side a similar convolute of hairs without a cyst." This, as far as I know, is the earliest reference to, even if not the earliest observation upon a fetal retrogression. But since rabbits are born naked, one is left to speculate upon the validity of this observation. However, that the idea holds is indicated also by some experimental work. Sokoloff's, '96, meager report, for example, seems to indicate that in dogs bilateral ovariotomy leads to the death of the embryo and to abortion of it and of the entire conceptus. Strahl and Henneberg, '02, also found that conceptuses in different stages of retrogression occur quite commonly among normally developed ones in the ferret, marmot and mole. They also found that the entire placenta and probably also the fetal membranes, as stated by Hubrecht, normally are retained for some time, even up to a month after parturition in the mole. Similarly, Frankel, '03, was able to cause the death and also the uterine absorption of conceptuses in rabbits up to the 20th day of pregnancy, through destruction of the corpora lutea. Frankel found that after 14 days, all that remained in the way of evidence of some pregnancies was an anemic ring which disappeared completely within three weeks. Henneberg,

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[1] From the Department of Embryology of the Carnegie Institute and The Department of Anatomy of Stanford University.

'03, also found that intrauterine death and retrogression of the guinea pig fetus can be effected experimentally through various means, and according to Koebner, '10, not merely ova or young fetuses are absorbed, but even the bones of older ones disappear completely under experimental conditions in the rabbit. It would seem unlikely, however, that such could be the case in any but the very earliest stages in the development of the skeleton for a considerable degree of acidity would have to develop in order to make this possible. Since it is believed that the development of acidity not only is slow but also slight in embryonic tissues, Koebner's conclusion regarding the bones seems to be open to some doubt. Wiener, '05, also found that autolysis is very slight in tissues with an alkaline reaction and that a slight increase in acidity greatly accelerates it.

Fraenkel, '10, extended and confirmed his work done in '03 by a very large series of experiments touching various phases of the corpus luteum problem and among other things concluded that one corpus luteum can protect at least three ova sufficiently to insure continued development.

Although the ova found by Huber, '15, were very young, the significance of the facts would seem to be similar. Likewise Mall, '15, while writing "On the fate of the human embryo in tubal pregnancy," stated that "we have no data on the number of ova which disintegrate early, but the study of comparative embryology warrants the conclusion that many young ova degenerate and disintegrate. I am informed by Dr. Huber, who has studied with great care much material among rats, that some of the fertilized ova break down before implantation. The same seems to be true regarding the pig. We usually find more corpora lutea in the ovaries than embryos in the uterus, indicating that all of the ova do not produce normal embryos." Similar phenomena also were reported by Meyer, '17, in regard to young conceptuses in the guinea pig. Curtis, '15, also reported the absorption of ova in guinea pigs in consequence of the injection of extracts of the human placenta, but unfortunately did not give convincing evidence regarding his knowledge of the presence of pregnancy in the animals concerned, and did not make a microscopic examination. Since Curtis stated that injections of defibrinated human blood had the same effect as the injection of extracts of the human placenta, and that the injection of extracts of guinea pig placenta and of guinea pig blood had no effect whatever, one can not help but feel decidedly skeptical regarding the trustworthiness of his experimental proof.

It is well to remember in this connection that phenomena which occur in other mammals, or conclusions drawn from these phenomena, must be used with caution when referred to man. The uteri of other mammals well may show a greater tolerance to the presence of dead fetal tissue and also greater resorptive power. Robinson, '04, stated that Vernhout showed that maternal tissues are not shed at the time of birth in the mole, and that some of the fetal tissues are retained to be absorbed later. Hill, it seems, found the same thing to be true in *Perameles* and *Dasyurus.* Jenkinson, '13, also stated, that in *Perameles,* the allantois and its blood vessels regularly are absorbed through the agency of maternal leucocytes by the parturient uterus and that fetal tissues are absorbed somewhat similarly in *Dasyurus.* However, since the young of some marsupials are in a very immature state during the first months of gestation and are then transferred to the marsupium as naked little fetuses said to be only about one inch long in the kangaroo, it is clear that absorption of the secundines at this stage of development would be something quite different from their resorption at the end of the gestation period in other mammals.

Although we have considerable evidence regarding retrogression and the partial or even the total intrauterine absorption of conceptuses in various mammals, I have not been able to find any conclusive evidence in the literature regarding the occurrence of this phenomenon in man. It is true that cases of Frankel, '03, Polano, '04, Rosenkranz, '03, and also cases reported by others are referred to as such examples, but a careful examination of the reports shows that those cases hardly can be regarded as falling under the head of intrauterine absorption of ovum, embryo or fetus. It is true that in the cases of Polano and Rosenkranz, the skeletal elements only were seen to have been discharged, but in the case of Polano the amniotic fluid nevertheless may have carried a great many fragments of embryonic tissue away with it beforehand. Furthermore, Polano does not claim his case as one of complete intrauterine absorption but merely as one of remarkable intrauterine maceration under aseptic conditions.

The history of the case of Rosenkranz, on the other hand, shows quite clearly that the fetus was destroyed by putrefactive changes. Rosenkranz himself emphasized this, but strangely enough ruled out entirely the occurrence of maceration. Rosenkranz further stated that the patient herself noticed the discharge of some bones with the "menstrual" flow. However, under the above conditions the recurrence of true menstruation is exceedingly unlikely unless the fetus was dead a considerable period before the rupture of the membranes occurred, and the placenta had been detached at least partly, for only under such circumstances could considerable regeneration of the mucosa occur and thus make the return to normal menstruation possible. It nevertheless is possible, however, that the time of abortion was co-incident with the date on which menstruation might have recurred. A small number of cases in the Mall Collection give a history which makes such a suggestion probable. Just why the expulsion of a dead, retained conceptus should occur at the time when menstruation would have recurred normally had pregnancy not supervened, is difficult to say, but since the inhibitory effect upon the menstrual cycle exercised directly or indirectly, by the living fetus is absent in cases of premature death and retention, it is possible that the abortion might occur at a time when the impulses of a return to the normal nonpregnant status of the maternal organism becomes more evident; that is, at the time of the recurrence of the normal menstrual cycle.

One can not but recall in this connection another group of cases which give a history of uninterrupted menstruation throughout the entire period of pregnancy. In some of these cases it is evident that it is a question of more or less regular hemorrhage rather than of true menstruation, and it may be possible that in the others these hemorrhages happened to fall at intervals of the same length as the normal inter-menstrual periods. In the last group of cases the exact status can not be discerned from the histories alone. However, since there is no endometrium or at most a partial one, to shed, genuine menstruation manifestly can not occur throughout pregnancy. It seems more probable that the cases of supposed uninterrupted menstruation fall into the last group although it is not impossible that rarely hemorrhage may occur only at the dates of normal recurrence. This would seem possible because of the defective inhibition of the returning normal impulses exercised by the dead or dying conceptus and the retrogressing corpus luteum. This conclusion harmonizes with the tendency to abortion which seems to be present at the time of periodic hyperemia and hyper-irritability in some of these cases.

Although dissolution of the embryo or fetus alone has long been known to occur, there seems to be no convincing evidence in the literature for the occurrence of intrauterine autolysis and absorption of entire conceptuses. Moreover, as already stated, it is to be doubted very seriously whether complete intrauterine absorption is possible after the formation of the skeleton has been well begun. The fetal parts at this time are so resistant that the uterus is stimulated to expel the macerated remnants long before absorption of them can occur. Furthermore, complete absorption would also seem to be hindered by the physical conditions which obtain in the human female. Only as long as the decidua capsularis is relatively thick, and hence effectively prevents the escape of the products of autolysized embryonic tissue, would complete absorption of a young uterine conceptus seem to be possible. The same thing probably is true in some cases of tubal pregnancy although the occurrence of early and repeated, yes, even prolonged, hemorrhage in them makes complete intra-tubal absorption much less likely. However, that complete intrauterine absorption actually does occur in the human female, it is the object of this article to establish.

The occurrence of missed abortion and also of missed labor long have been matters of common knowledge among physicians. But these are phenomena usually connected with the later months of pregnancy. In the overwhelming majority of these cases the fetus was retained for a considerable period after its death and then aborted in a more or less macerated condition. Under these circumstances it may, to be sure, undergo absorption in part, but expulsion of macerated or even calcified remnants of the fetus nevertheless eventually occurs. That this is so even after an exceedingly long period of retention in utero, is shown splendidly by the case of Schaeffer, '98.

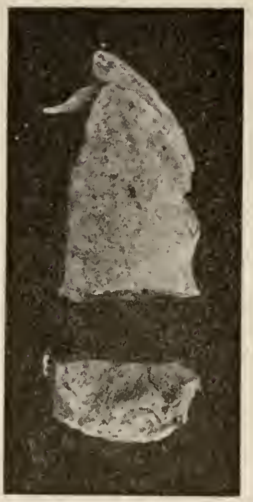

FIG. 1. External appearance of No. 698. The excised portion was used for microscopic examination. × 1.

Among the large series of over 2,000 abortuses in the Mall Collection, I have so far found only a few specimens of almost complete intrauterine absorption. In one of these, number 698, only a few vestiges of syncytium and trophoblast remain. Not a single fragment of the chorionic or amnionic vesicle or of the embryo could be found upon microscopic examination. Were it not for the presence of decidua and the above microscopic embryonic remnants, one might doubt whether pregnancy really had supervened in this case. However, since the specimen shown in cross section in Fig. 1 was aborted with the entire intact decidua which still surrounded the remnants of the conceptus completely as shown in Fig. 2 there manifestly could have been no loss of embryonic tissue either before or during abortion. This abortus, which measured 50 x 20 x 13 mm. was donated by Dr. N. E. B. Iglehart, of Baltimore. It had a menstrual age of 56 days, but the condition of the few remnants and the size of the implantation cavity show that development did not proceed very far before growth was inhibited. Aside from the absorption of almost the entire conceptus, the decidua not only is infiltrated, but also shows degenerative changes. As illustrated by Fig. 2, which shows the intact capsularis separated by a narrow space from the vera, the former is filled completely by blood clot. It is at the periphery of this clot that the isolated microscopic remnants of the syncytium and trophoblast, together with a few gossamer or shadow villi are found. Since there is no blood between the capsularis and the vera it would seem to follow that the hemorrhage was limited entirely by the capsularis, a conclusion which is indicated also by the absence of a history of bleeding. Since the last menstruation began on April 11 and the abortion occurred on June 6, it is seen that the latter occurred on the first day of the beginning of the second lapsed menstrual period. Although the appearance and the condition of the decidua seems to suggest that considerable regeneration of the endometrium had occurred, it is possible though unlikely, that the bleeding accompanying the abortion was

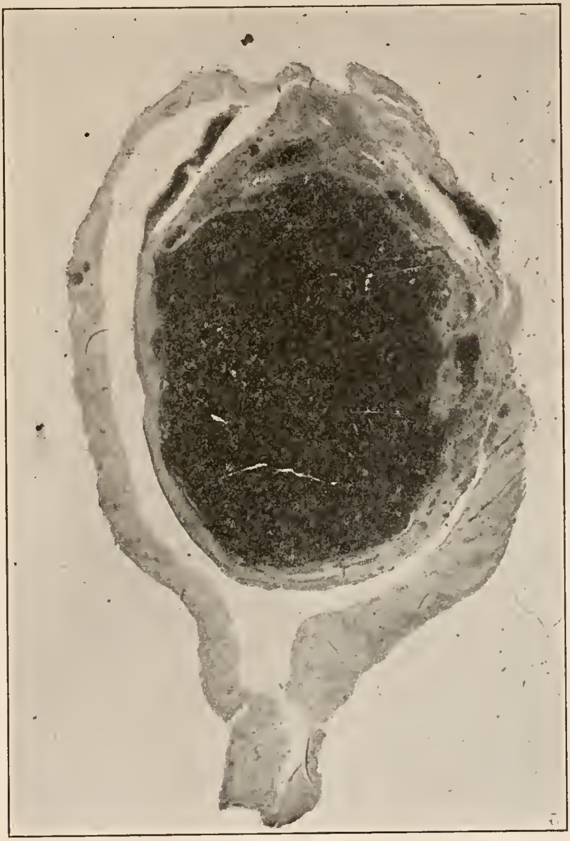

FIG. 2. Cross section of the above showing the disposition of the decidua and the contained blood clot. × 5.

menstrual and that hence the abortion should be regarded as a mere incident accompanying the return of normal menstruation rather than as the predominating event.

That considerable restoration of the endometrium may occur while the conceptus still is within the uterus was shown by the case of Orloff, '95. In this case the endometrium was composed of a cylindrical epithelium and the uterine musculature showed no evidences of the presence of gestation changes. Ivanoff, '98, also found the decidua absent in a case of long retention and its place taken by a low cylindrical epithelium although the placenta still was partly attached to the uterus. Frankel, '03, also emphasized the fact that regeneration of the endometrium may begin before abortion occurs and these things make it possible that hemorrhage which may occur at time of abortion may largely be true menstrual hemorrhage.

The absence of blood between the capsularis and the villi and the absence of a history of bleeding do not, to be sure, imply that the development of this conceptus progressed uninterruptedly until birth. The histologic picture alone is conclusive proof to the contrary. In the absence of any larger portion of the conceptus it is impossible to say about how far development had proceeded, but it is unlikely that it proceeded much beyond the first month. In any case, disintegration, solution and resorption of almost the entire conceptus surely must have consumed several weeks at least. Indeed, it is possible that the ovum never became firmly attached, though imbedded in the decidua.

In other cases it also seems likely that the fertilized ovum became inbedded quite normally but that it was strangulated by severe hemorrhage which loosened the attaching villi, thus interrupting the intervillous circulation. Since the resulting stagnation of the blood must make the indispensable chemical interchanges upon which the life of the embryo depends, impossible, the latter probably dies first. It is decidedly interesting that considerable hemorrhage, sufficient, in fact, to result in the death of both the embryo and the chorionic vesicle, can occur while the whole conceptus still is surrounded by the decidua capsularis, without rupture of the latter. The failure of absolute or complete absorption of the last few small remnants of this conceptus probably may be attributed to the fact that the small remnants of degenerate trophoblast and syncytium which remain, or the influence of the corpus luteum no longer were able to inhibit menstruation. Hence the decidua, together with these few small remnants of the conceptus were expelled *in toto* and it may be extremely significant that this occurred exactly two menstrual months after the beginning of the last period. Since three other specimens of a series of seventeen composed of villi only were aborted at the time of recurrence of the regular period, the idea that abortion occurs oftener at this than at any other time would seem to receive some confirmation. Moreover, it would seem quite natural that a detached decidua which has subserved its functions would be more likely to be shed at this time and that an unabsorbed conceptus which had been converted essentially into a foreign body, should then also be expelled. Since detachment of the decidua also permits regeneration of the mucosa and isolates the conceptus, it removes the inhibitory effects of the conceptus upon the maternal organism and clears the way for a return to the normal non-pregnant status.

It, to be sure, is impossible to decide how far the development of this conceptus had progressed before its death, but the marked extent of the absorption shows beyond doubt that the latter would have been completed long before the advent of the next or third menstrual period had the second period also been inhibited. Under these circumstances the empty decidual cast then would have been expelled alone and well might have directed attention to the possibility of the existence of a tubal rather than a uterine pregnancy.

A second case is number 970, donated by Dr. R. W. Hammack, of Manila. This specimen is interesting not only because it also is a case of marked intrauterine absorption, but because it was obtained with the entire uterus, at necropsy. The chorionic vesicle, which measured only 3 x 5 mm. together with the entire thickness of the decidua and the musculature, is shown in cross section in Fig. 3. The uterine cavity contained some blood and the entire decidua was covered with hemorrhagic nodules the largest of which were about 10 mm. in diameter. One of these which was a trifle larger than the rest contained the conceptus. The cavity of the chorionic vesicle was filled with a homogeneous substance containing degenerate cells and portions of disintegrated chorionic membrane. The villi are only about .5 mm. in length and are covered with trophoblast and syncytial buds.

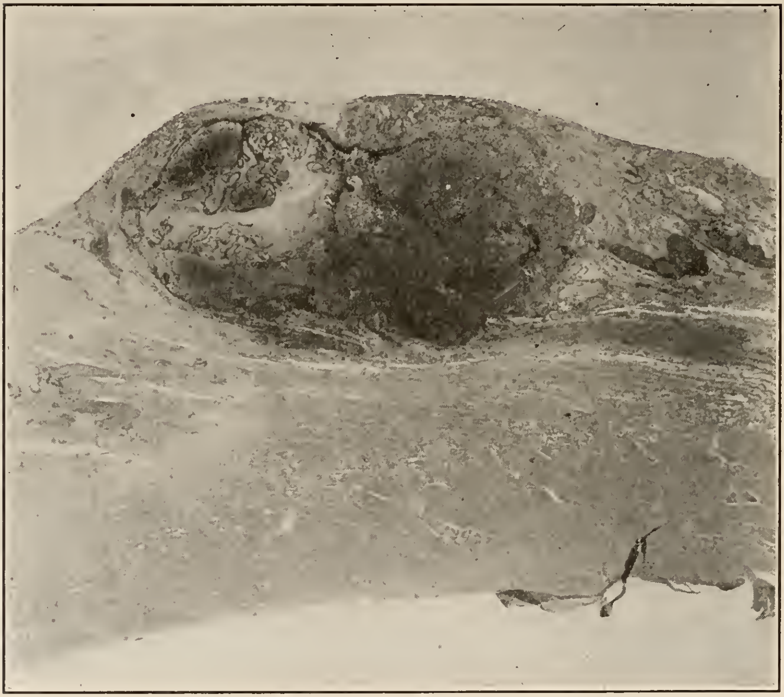

FIG. 3. Sections through the decidua and conceptus No. 970, showing a slightly bulging but intact capsularis.

No trace of the embryo or of the amnion was found, although the whole conceptus is covered still by the capsularis, which, like the rest of the decidua, is infiltrated. Although lysis and absorption did not progress so far in this as in the previous specimen, the process nevertheless is far advanced. It proceeded no further probably because the mother committed suicide, although the multiple hemorrhages in the decidua would seem to suggest that abortion nevertheless was seriously threatened, if not inevitable, before she took her life. That the focal hemorrhages in the decidua could be attributed to the hydrochloric acid swallowed with suicidal intent, is extremely doubtful, for the histologic condition of the conceptus shows conclusively that the changes in it could not possibly have been produced in the short interval of four days which elapsed between the suicidal attempt and death. Since the menstrual history of the case remains unknown it is impossible to determine the menstrual age of the specimen but the degenerate chorionic vesicle would seem to imply an age of only about ten or twelve days. However as this young woman was but sixteen years and apparently illegitimately pregnant, it is more than likely that the suicidal attempt occurred, as so often is the case, during the time of the first lapsed period. Hence the surmise that the multiple hemorrhages in the decidua may have been provoked by the returning menstrual cycle, gains somewhat in probability. Especially so since the size and the condition of the conceptus both suggested that it must have died some weeks previous to its abortion.

A third early specimen illustrating the progress of intrauterine absorption is number 962, donated by Dr. Joseph M. Jackson, of Pittsburgh. In contrast to the preceding two, this chorionic vesicle contained a macerated embryo 4 mm. long. The menstrual age is unknown but the chorionic vesicle measured 34 x 28 x 24 mm. and was covered almost entirely by villi. The latter which contained degenerating vessels, are matted together with necrotic trophoblast and show other evidences of retention. As shown in Fig. 4, which represents a cross section of the entire conceptus with the surrounding decidua, the amnion was preserved and contained some coagulum. Mall found the embryo greatly macerated and the organs and cavities partially obliterated. The slight break in the decidua capsularis may be the result of handling or of technical procedures. Since the specimen was aborted with the entire decidua there can be no question of escape of a portion of the conceptus.

That it not alone is very young conceptuses which may undergo almost complete lysis, is illustrated by number 606, a chorionic vesicle measuring 18 x 13 x 18 mm. This specimen, which was donated by Dr. Charles S. Parker, of Baltimore, is covered with villi 2.35 mm. long. Yet Mall found no trace of an embryo and stated that the villi and the chorionic membrane are structureless in spite of the outwardly normal appearance of the chorionic vesicle. In the absence of the clinical history one must needs be cautious but I think it safely can be assumed that in this case neither embryo nor amnion disappeared solely as a result of postpartum maceration. That this assumption probably is correct is shown also by other specimens, the histories of which fortunately are known. Nevertheless, maceration, although not necessarily putrefactive maceration, undoubtedly was an important factor in the production of the state in which this specimen is found. This conclusion is confirmed by the occurrence of all manner of transitions between the almost perfectly preserved structure and the pure shadow or gossamer pictures

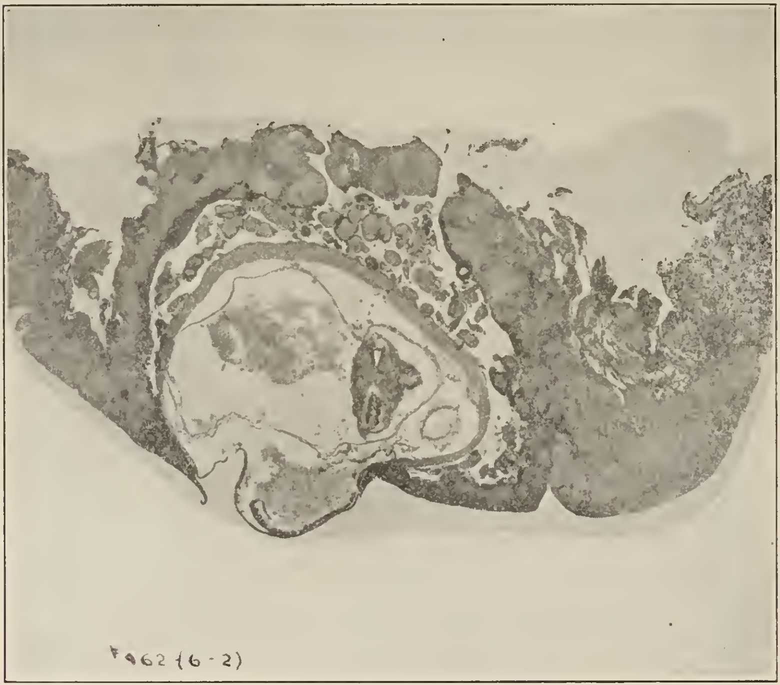

Fig. 4. Section through the decidua and the contained conceptus No. 962. The capsularis is slightly broken.

such as presented in the photograph of the cross sections of the villi of this specimen shown in Fig. 5. All that remains of the villi is a spiderlike web, the fibres of which are exceedingly fine but which nevertheless preserves the form of the villi and of the chorionic membrane so perfectly that Mall especially recorded that the external appearance of the chorionic vesicle was normal.

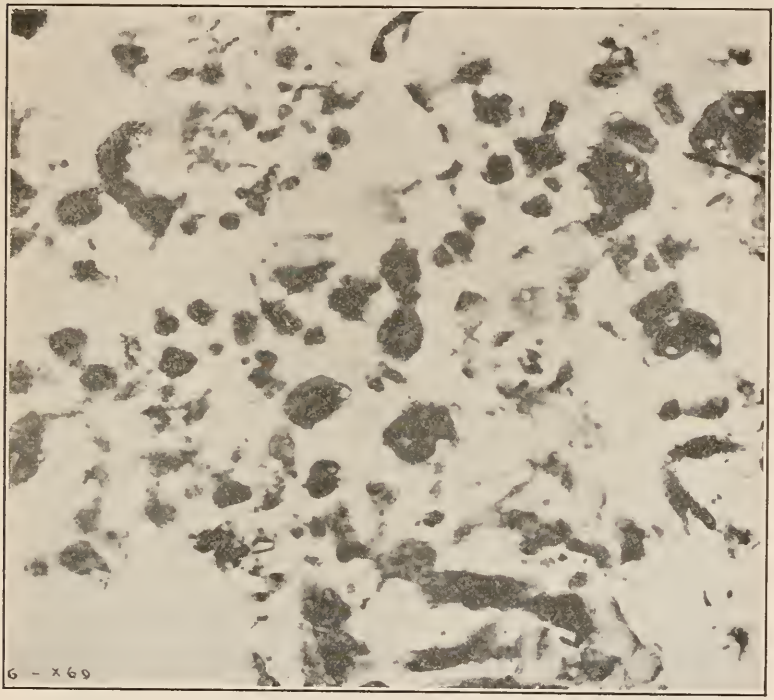

FIG. 5. Cross-sections of the gossamer villi of No. 606. × 69.

Since chorionic vesicles devoid of an embryo when examined form about 32 per cent. of those classed as pathologic in the Mall Collection it is evident that absence of the embryo itself is relatively common in early abortuses. It would be incorrect, however, to assume that they had undergone absorption in all these cases. A fine example of one of these empty chorionic vesicles is number 1224, a portion of which is shown in Fig. 6. This specimen was found in an unopened uterus removed at hysterectomy for cervical myoma. The chorionic vesicle, which measured 36 x 25 x 13 mm. was collapsed, free from the uterus, and imbedded in coagulum. The only content of this chorionic vesicle was a dark grayish coagulum which contained no remnant of the embryo or of the amnion. This almost amorphous so-called magma included only a few isolated cells. In spite of this fact the trophoblast, which had markedly proliferated, was well preserved over large areas, and many of the vessels in the chorionic membrane were filled completely with erythroblasts. A few degenerate masses of trophoblasts and fused degenerate villi also are present. Some villi show evidences of

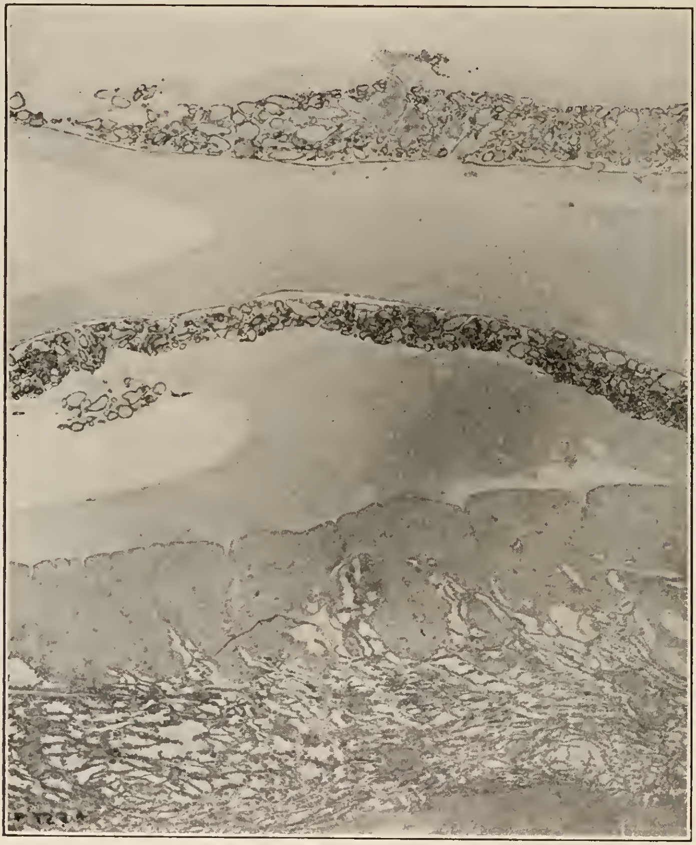

FIG. 6. Section through the isolated empty conceptus and the adjacent decidua of No. 1224.

maceration, others of "mucoid" degeneration although they still may contain vessels. Some, however, are represented by a hyaline outline only. The stroma and the epithelium of many of the villi are well preserved, however, and the same thing holds for the chorionic membrane. The decidua shows slight general and very marked local infiltration. Some remarkably dense periglandular and perivascular zones of infiltration also are present. The mucosa, too, is infiltrated and contains islands composed exclusively of round cells. Besides maceration effects, many of the villi show marked changes, undoubtedly hydatiform in character. In this case it is possible that we are dealing not so much with absorption as with dissolution of the embryo, for the digestion products of embryo, yolk sac and amnion, instead of having been wholly absorbed still may be contained in the fluid within the chorionic vesicle.

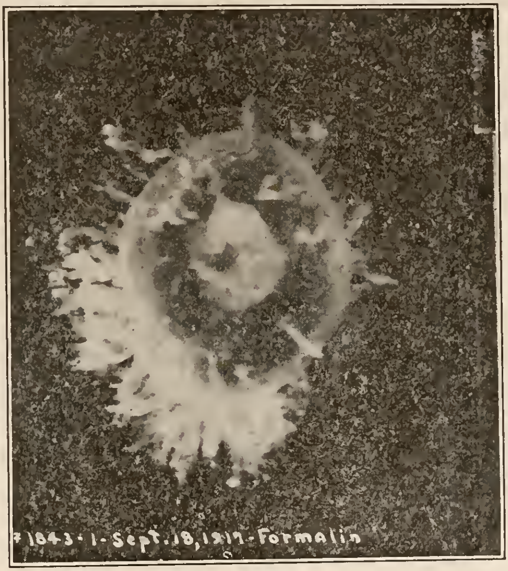

FIG. 7. External appearane of No. 1843. × 6.

Before briefly considering the evidence regarding absorption offered by tubal and ovarian specimens, I wish to refer to number 1843. This specimen, which was donated to Stanford University by Dr. Eugene V. Falk, of Modesto, California, had the villi rather sparsely and irregularly distributed as Fig. 7 shows. However, the entire specimen was so splendidly preserved that investigators of unique opportunity and experience were uncertain as to its normality. Even after careful inspection under low magnification the writer, too, felt uncertain, but on receipt of the specimen he had informed Dr. Falk that it probably was pathologic. This opinion was based almost wholly on the irregular distribution of villi, their complete absence on part of the surface; on the large size of the yolk sac; the unusual translucence of the entire specimen; and upon the *apparent* absence of the embryonic disc. About one-third of the entire surface of the chorionic vesicle was devoid of villi and where they were present they seemed to be in widely different stages of development. They differ markedly not only in length and in diameter but also in the complexity of their branching. Some which were represented by fine threads merely were found to be represented by stroma only, the epithelium having been stripped, probably during the removal of the blood in which the specimen was imbedded before it was received at the laboratory. Other villi are torn, those which are preserved are clubbed but slightly and some are so short that they look like little droplets on the surface of the chorionic membrane. Those near the bare areas are almost transparent but nearer the other pole they become more opaque. The caliber varies from 1/6 to 1 mm. and the greatest length is 2.25 mm. The chorionic vesicle measured 6 x 4 x 5 mm. and the partially invaginated yolk sac, 2 x 2.6 x 1.8 mm. A smaller, less transparent vesicle, which was thought to represent the amnion, was seen between the yolk sac and the chorionic membrane, but no embryonic disc could be recognized. An examination of the microscopic sections of this vesicle showed that it was macerated. The epithelium is missing in many places and the histologic details are wanting. Hence this chorionic vesicle very evidently ceased to live some time before it was aborted, and this conclusion is corroborated also by the clinical history. The menstrual age of the specimen is 39 days but the chorionic vesicle, which was approximately spherical, measured only 6.5 mm. instead of 25 mm. to 30 mm. as implied by the menstrual history. That growth ceased long before the occurrence of the abortion is implied also by the fact that Dr. Falk stated that the patient had a slight uterine hemorrhage about 10 or 12 days before the abortion. It is interesting, and probably also significant that the date of this hemorrhage also coincides with the time when the first lapsed period was due. This is possible because this conceptus apparently had been dead sufficiently long to fail to inhibit the return of menstrual bleeding. Since the size of the chorionic vesicle suggests an age of 10 to 12 days, this assumption seems warranted, especially since it may be assumed that development probably never proceeds undisturbed to the time of abortion, whenever the pregnancy is terminated spontaneously or perhaps better, without the intervention of external or of internal mechanical forces or factors It may be largely because of this fact that conceptuses from abortions resulting from intrauterine causes always are macerated.

Careful examination of the serial sections generously made in the Carnegie Laboratory of Embryology, fails to reveal any remnant of the body of the embryo except perhaps a small nodule shown above (2) in Fig. 8. Yet according to the menstrual age the embryo should be 10–12 mm. long. The appearance of this nodule suggests that it may be a remnant of the primitive streak in spite of its deep location, although it may also be a rudiment of the allantois. The yolk sac is large and invaginated and its size out of all proportion to that of the embryonic Anlage. Between the latter and the chorion there is a rather large mass of cells containing a space which I take it, represents the amniotic cavity. An examination of this cavity and of the surrounding cells suggests that it resulted from splitting as is the case in bats and as is assumed for man. The cavity, to be sure, may have resulted from dissolution of the cells *in situ* and if in fact it represents the early amniotic cavity, then it is certain that whatever its genesis, it was not formed in consequence of folding. It would seem then that we have here a conceptus in which the process of development of the embryo itself was inhibited very early and that the yolk sac and the chorion continued to grow for some time.

Aside from showing a probable and hitherto unobserved stage in the formation of the amniotic cavity, this pathologic chorionic vesicle is of special interest also in the absence of coagulum or so-called magma. The chorionic vesicle and the space taken for the amniotic cavity were filled with perfectly clear fluid. Although this small conceptus is markedly macerated it is only in the early stages of disintegration. Nevertheless, much longer retention might have resulted in its complete dissolution even if not its complete absorption. Since this small vesicle was aborted in its entirety with blood clot, it is highly probable that most of these degenerative changes occurred after it was loosened from the implantation site, probably by rupture of the decidua capsularis in consequence of hemorrhage.

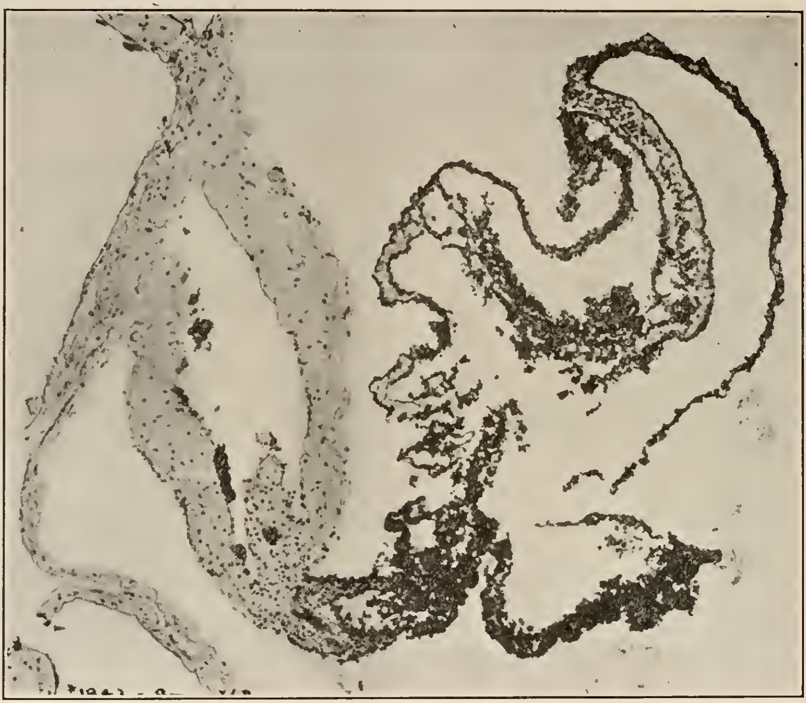

FIG. 8. Section through the chorion, amnion (?) and yolk sac of No. 1843 at the point of attachment. The small nodule of cells above (2) may be a remnant of the primitive streak or of the allantoic stalk. × 69.

In other older conceptuses in earlier stages of disintegration, as number 2047, the amnion is preserved and distended as shown in Fig. 9. Both chorion and amnion are macerated and distended with clear fluid and it seems strange indeed that the embryo and the yolk sac may disappear completely without even a final clouding of the amniotic fluid. There may have been temporary clouding, but every now and then a specimen is received in which both the vesicles are distended with absolutely clear fluid, a fact which also implies that autolysis of the embryo occurred without digestion of the enveloping amnion.

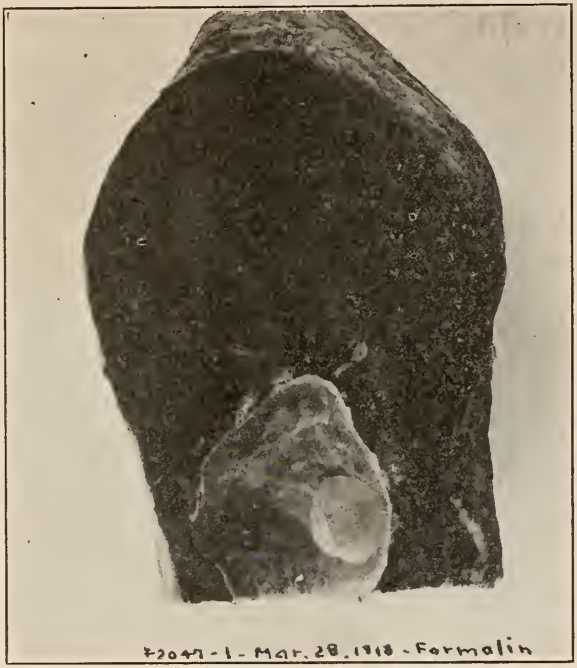

Fig. 9. Gross appearance of abortus No. 2047 showing a large blood clot containing a transparent chorionic and an empty amniotic vesicle. X 3.

Since only a pseudo-decidua forms, and then but rarely and very early, in cases of tubal pregnancy, the sequence of events leading to dissolution of the conceptus must be rather different. There can be no accumulation of blood in a capsularis. Hence the conceptus usually becomes isolated in blood or blood clot within the tube, and undergoes degeneration as the hemorrhage continues and the tube becomes distended. If detachment of the conceptus occurs very early, it is conceivable that it may undergo complete disintegration also within the tube, that the latter may heal and the symptoms subside completely. While the occurrences of such instances of spontaneous cure of tubal pregnancy undoubtedly are exceedingly rare, evidence at hand seems to show that they can not be wholly excluded. This, I am told, agrees also with contemporary clinical opinion.

The possibility of tubal abortion with subsequent intraperitoneal disintegration, lysis and absorption must, to be sure, also be borne in mind. The occurrence of nothing but fragments of villi in a tubal clot as shown in the case of number 977 represented in Figure 10 cannot be accepted as positive proof that all the rest of the conceptus was absorbed intratubally. A portion may have been aborted, yet specimens such as number 2035 and 1938 speak eloquently for the *possibility* of absorption. The embryo and yolk sac in the latter specimen are disintegrated almost completely and the scarcely recognizable remnants lie isolated in the chorionic cavity which is moderately filled with an amorphous coagulum. The stroma of the chorionic membrane is œdematous and degenerate but nevertheless contains some well-preserved vessels, a few of which contain some blood cells. The same thing is true of the stroma and of the vessels of the villi which also are in process of dissolution. A moderate amount of trophoblast is present but there is very little syncytium. The epithelium of some of the villi has undergone hyline degeneration. The blood cells in the large clot in which this chorionic vesicle, which measured 8 x 5 mm. in section, was imbedded, are preserved fairly well, especially near the vesicle. Nevertheless, the old conceptus very apparently is in a state of disintegration and lysis, the tube wall is very thin and the mucosa congested, hemorrhagic and atrophic.

Number 2035, also a tubal specimen, likewise is an empty chorionic vesicle in process of disintegration, and many other specimens might be listed, but these examples suffice to indicate that intratubal, as well as intrauterine lysis and in part at least of absorption, of conceptuses undoubtedly occurs in the human being. It is of course exceedingly unlikely that in case of the uterus this can occur before the impregnated ovum is imbedded, for failing to imbed it undoubtedly would escape relatively promptly although the observations of Kirkham, '16, show that the fertilized ova in mice suckling their young, may lie unimplanted within the uterus for over a week.

A free fertilized or unfertilized ovum which disintegrated within the tube on the other hand, might be absorbed completely. The same thing holds for early conceptuses within the implantation cavity, for a relatively small amount of hemorrhage could detach them completely without rupturing the capsularis. Death, disintegration, and absorption, as illustrated in the cases above, then might occur. In case of older specimens the hemor-

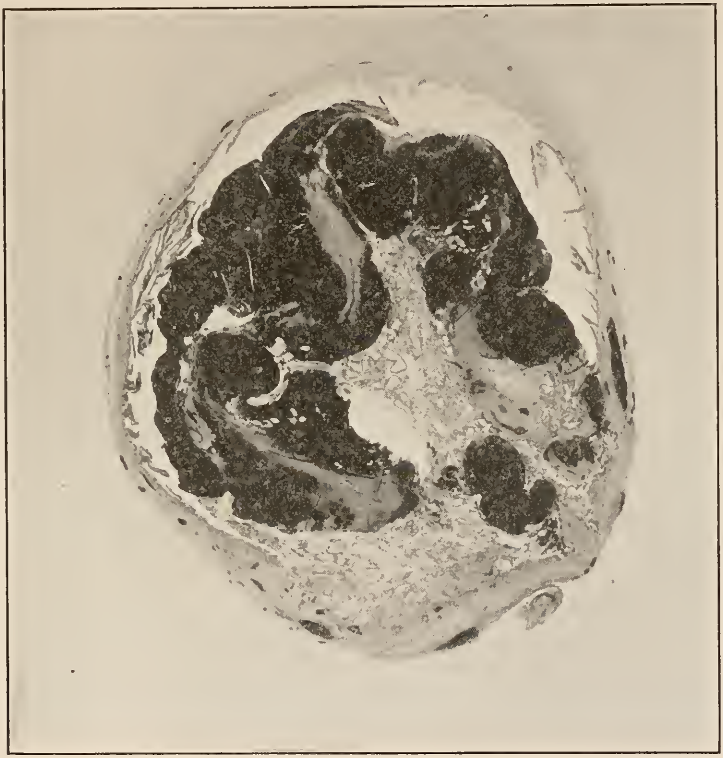

FIG. 10. Cross section of a tube with the contained blood clot and scattered chorionic villi, No. 977. × 4.4.

rhage responsible for the loosening of the conceptus would have to be proportionately much greater for the attachment of the placenta is firmer though rupture of the capsularis easier. That this assumption is correct is shown also by the almost universal history of bleeding in these cases and it is only in the early stages of development that the conceptus can be expelled entire while still contained in the implantation cavity, and aborted completely wrapped in the decidua as in case of number 698.

Since 12.8 per cent. of all abortions and 32.3 per cent. of all those classed as pathologic in the first one thousand accessions in·the Mall Collection are composed of villi only, of empty chorionic vesicles, and essentially of chorion and amnion, one might assume that all these specimens represent stages in the process of intrauterine disintegration and absorption. Most of them undoubtedly do belong in this category but in many cases in which villi only are found the rest of the conceptus and in others the embryo as well, has been lost. Since 46.4 per cent. of all tubal specimens and 71. per cent. of all those classed as pathologic are composed of villi only, of empty chorionic vesicles and of chorion and amnion only, it might be assumed that digestion and absorption are more active within the tube than the uterus. Such a conclusion is not justified however, for almost all tubal specimens are isolated while young and only exceptionally does one reach the later months of pregnancy. Hence those falling in the above-named three groups of tubal specimens form a relatively larger percentage.

Cases of partial dissolution of the embryo are of course common, as almost every one knows. As far as I can learn however, the two cases reported here are the only ones offering unequivocal proof that dissolution of the entire conceptus may be absolutely complete and that the intact empty decidua then may be aborted. Such an event could for various reasons probably occur only in the early stages of pregnancy and must undoubtedly be relatively rare. Nevertheless, I am convinced that careful examination of all material aborted will multiply the evidence. From these things it also would seem possible that rarely pregnancy may supervene and be terminated without having attracted any attention whatsoever.

The phenomenon of intrauterine lysis is interesting also from a chemical standpoint. What the antomist would like to know is not merely in what respects the composition of the intra- and peri-amniotic fluids has been changed, but just what the enzymes are that have caused a complete lysis of the embryo; where these first arise and act; and how and why they become active. These, and many other questions the chemist only can answer. For this answer fresh material is indispensable, but this the neighboring practitioners or a closely associated clinic can supply. That the lysis of these early embryos and undoubtedly also of the chorionic vesicles is not due primarily or even very materially to phagocytic activity is very evident even upon cursory examination. In the presence of the intact chorionic and amnionic vesicles in some specimens, such a process is wholly excluded. Besides, one never sees any evidence of phagocytosis of the preserved fetal by the maternal tissue in human conceptuses. Evidences of the contrary process are not wanting.

That the embryo or fetus usually is the first member of the conceptus to disappear has already been implied by stating that in 32 per cent. of the abortuses grouped as pathological in the Mall Collection, the embryo is missing. Although the absence of the embryo does not necessarily mean, in every case, that it underwent complete autolysis, this no doubt is true in by far the great majority of these cases. The fact that the embryo disappears first may be due to a lower resistance on its part than that possessed by either amnion or chorion, or to a preponderance of enzymes within it. Autolysis of the body of the embryo before that of the membranes, also may be due to the fact that the adnexa, especially the chorion, or at least parts of it, often preserve vitality long after the death of the embryo because of the direct relation to the uterus. Nevertheless, it often is surprising how long the form of a small retained fetus or even of the amnion apparently has been preserved, although it should be remembered that in some cases the preservation of external form gives little indication of the true state of the constituent tissues. If the advent of proteolytic fat-, and carbohydrate-splitting enzymes in fetal tissues is gradual, the surprisingly long time during which some of the small embryos are preserved may be due in a considerable degree to this fact. Jones and Austrian, '07, found, for example, that the livers of young pig embryos contain no nulein enzymes, but that these increase as fetal development proceeds. The latter finding seems to be confirmed also by data given by Mendel and Leavenworth, '08, although these investigators found enzymes present at all ages. Schlesinger, '03, who was, I believe, the first to establish the occurrence of autolysis in retained human fetuses, also found that it varied with age and other conditions.

Since the chorionic villi do not undergo autolysis first, the conclusion that heterolysis is not an important factor in lysis of the conceptus, also seems justified. If enzymes of decidual origin played any large part in the process of lysis of the conceptus, one might, I presume, expect to find instances in which at least portions of the chorionic vesicle underwent lysis although the embryo itself remained quite unaffected. This, however, never seems to be the case and the same thing holds for the placenta and the chorionic vesicle and also for the decidua. That heterolysis probably plays only a very subsidiary rôle is indicated also by the fact that autolysis takes place in tubal implantations as well, and still more convincingly by its occurrence in ovarian implantations, as illustrated especially by the case of Holland, '11, and by other cases referred to by Meyer, and Wynne '19, in which the escape of portions of the conceptus from the ovarian implantation cavity could be wholly excluded. One reason for prolonged preservation of the decidua no doubt lies in the fact that it as a whole or at least in large part retains vitality because it remains quite undisturbed in its vascular relations.

REFERENCES.

Curtis, A. H.

'15 Experiments in the Production of Abortion and Labor by use of Placental Extracts. Surg. Gyn. and Obst., Vol. 20.

Fraenkel, E.

'03 Ueber "missed labor" und "missed abortion." Samml. klin.Vorträge, Volkmann N.F. Gyn.

Fraenkel, L.

'03 Die Function des Corpus luteum. Arch. f. Gyn., Bd. 68.

Henneberg, B.

'03 Experimentelle erzeugte Rückbildungsvorgänge am graviden Säugetieruterus. Anat. Anz., Bd. 24.

Holland, Eardley.

'11 A Case of Ovarian Pregnancy; Probably Bilateral. Jr. Obst. and Gyn. Brit., Emp., Vol. 20.

Huber, G. Carl.

'15 The Development of the Albino Rat *Mus norvegicus albinus.* II. Abnormal Ova; End of the First to the End of the Ninth Day. Jour. Morph., Vol. 26.

Iwanoff, N. K.

'98 III., Ein interessanter Fall von "missed abortion." Centralbl. f. Gyn., Bd. 22.

Jenkinson, J. W.

'13 Vertebrate Embryology.

Jones, W., and C. R. Austrian.

'07 On the Nuclein Ferments of Embryos. Jr. Biol. Chem., Vol. 3.

Kirkham, William B.

'16 The Prolonged Gestation Period in Suckling Mice. Anat. Rec., Vol. II.

Koebner.

'10 Knochenresorption bei intra-uterinen Eischwund. Arch. f. Gyn., Bd. 91.

Mall, F. P.

'15 On the Fate of the Embryo in Tubal Pregnancy. Contributions to Embryology. Vol. I., No. 1, Washington, D. C.

Mendel, L. B., and Leavenworth, Charles S.

'08 Chemical Studies on Growth. V., The Autolysis of Embryonic Tissues. Am. Jr. Phys., Vol. 21.

Meyer, A. W.

'17 Intra-uterine Absorption of Ova. Anat. Rec., XII.

Meyer, A. W., and Wynne, H. M. N.

'19 Ovarian Pregnancy with Report of a Case. J. H. H. Bull., Vol. 30.

Müller, H.

'47 Abhandlung über den Bau der Molen, der medicinischen Facultät an der Universität Würzburg bei der Habilitation als Privat-docent vorgelegt. Bonitas-Bauer, Würzburg.

Orloff.

'95 Ueber einem Fall von langdauernder Retentio ovi in utero Prag. med. Wchnschr., Nr. 22.

Polano.

'04 Intrauterine Scelettierung. Zentralb. f. Gyn., No. 14.

Robinson, Arthur.

'04 Lectures on the Early Stages in the Development of Mammalian Ova, and on the Differentiation of the Placenta in Different Groups of Mammals. Jr. Anat. and Phys. Lond., Vol. 38, N.S., 18.

Rosenkranz.

'03 Skelettierung eines etwa viermonatlichen Fötus im Uterus infolge Fäulnis der Frucht bei missed abortion. Berliner klin. Wochenschr., Nr. 51.

Schaeffer, Oskar.

'98 Ueber einjährige Retention eines Abortiveis. Monatschr. f. Geburtsh., Bd. VIII.

Schlesinger, Eugen.

'03 Untersuchungen über die Abhängigkeit der autolytischen Prozesse von physiologischen und pathologischen Verhältnissen. Beitr. z. chem. Phys., Bd. 4.

Sokoloff, A.

'96 Experimentelle Beiträge zur Frage über die Wirkung der Eierstock Exstirpation auf die Schwangerschaft. Centralbl. f. Gyn., Bd. XX.

Strahl, H., and Henneberg, B.

'02 Ueber Rückbildungserscheinungen am graviden Saügetieruterus. Anat. Anz., Bd. 20 u. 21.

Wiener, Hugo.

'05 Ueber den Einfluss der Reaktions auf autolytische Vorgänge. Zentralbl f. Phys., Bd. 19.

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BIOLOGICAL BULLETIN

THE DEGENERATION OF YOLK GLANDS AND CELLS IN CESTODES.

R. T. YOUNG.

Yolk glands, while frequent, are not of universal occurrence in flat worms, nor do they apparently bear any constant relation to the systematic position of the various groups of this phylum. In the Acœla, which are probably to be regarded as the most primitive of the latter, they are lacking, with the exception of *Polychœrus*, while the same is true of the polyclads, which are one of the more specialized of these groups.[1]

Among trematodes they are universally present with the exception of *Gyrodactylus*, where their absence is possibly correlated to the peculiar mode of reproduction, while in cestodes they are typically present, though reduced in some and in one case at least absent.

Braun[2] in his description of the yolk glands in this class has pointed out their gradual reduction in size from those such as *Tetrarhynchus tetrabothrius*, in which the gland forms a mantel around the entire proglottid, to those such as many Tæniæ, where the gland is reduced to a small unpaired organ adjacent to the ovary. It is this reduction of yolk glands in some cestodes, together with certain corollaries deducible therefrom, that I wish to discuss briefly in this note.

In a study of gametogenesis in cestodes upon which I am engaged, I have examined the reproductive apparatus of a number of species, in one of which I am unable to discover a yolk gland, while in another I find evidence of its degeneration. The species in which this organ is evidently lacking is *Thysanosoma actinoides.* A yolk gland in this species was described by Stiles and Hassall in 1893.[1] Their work was done, however, on material which they expressly state was in such poor condition that many details could not be worked out. In a study of the reproductive organs in this form Swingle[2] was unable to find a yolk gland, nor can I do so. Therefore I believe that the former authors were misled, mistaking possibly a posterior lobe of the ovary for a yolk gland.

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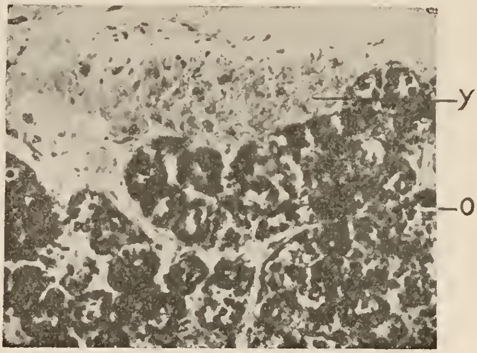
[1] This last statement holds good even though the polyclads be considered as directly derived from the ancestral form. If the Acœla however be regarded as degenerate, rather than primitive types, then the absence of a yolk gland here may be attributed to such degeneracy. This would not explain their presence in *Polychœrus* however, nor their absence in *Gyrodactylus*, which is exceptional among trematodes in this respect.

[2] Braun, M., "Cestodes" in Braun's Kl. and Ord. IV., Ib.

In *Hymenolepis* sp., I find evidence of the degeneration of the yolk gland, for here not only is the gland reduced in size, but it contains no yolk. Yolk takes iron hæmatoxylin with avidity, so that if present in the yolk cells it should appear with this stain, as it does very clearly in the ovary, whose cells are heavily laden with it. In the figure is brought out very clearly the distinction between the yolk filled ova and the empty yolk cells. This is true at least of later stages of the gland. In earlier stages the densely crowded and heavily staining nuclei



Yolk gland (*Y*) and ovary (*O*) of *Hymenolepis* sp. × 500.

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[1] Stiles, C. W., and Hassall, A., "A Revision of the Adult Cestodes of Cattle, Sheep and Allied Animals," U. S. Dept. Agric., Bur. An. Ind., Bulletin No. 4.

[2] Swingle, L. D., "The Morphology of the Sheep Tapeworm, *Thysanosoma actinoides,*" University of Wyoming, Agric. Exp. Station, Bulletin No. 102.

render it impossible to say that no yolk is present at this time. It is not evident at this time however nor is its presence to be expected in early stages of the yolk gland, before any considerable differentiation of its cells has taken place. The nucleus of the adult yolk cell apparently offers further evidence of degeneration for it is very irregular and broken in form and contains but little chromatin, though how much of this appearance is natural and how much artificial, due to imperfect action of the fixative on exceedingly plastic structures, I cannot say. In earlier stages however the yolk cell nuclei are fairly definite structures in similarly fixed material, so I incline to the belief that their irregular appearance in later stages is due to degeneration.

I have pointed out above that there is little constancy in the occurrence of a yolk gland in flat worms. It may exceptionally be present in some form in a group, where otherwise it is absent, and vice versa. The fact that it is almost certainly to be regarded as a specialized part of the ovary, and that its function may be largely assumed by the latter even when it is present, as in cestodes, reduces its functional importance and consequently its selective value, and may therefore increase its variability and render it more liable to degeneration.

In cestodes the function of the yolk gland has been largely supplanted by the ovary itself, and its absence in one, and apparent degeneration in another species may very likely be prophetic of its future degeneration in cestodes as a group, together with so many other organs.

A further point of interest in connection with these observations is the fact that in *Thysanosoma actinoides*, where a yolk-gland is absent, the cell so frequently attached to the ovum and embryo, which has been assumed by several writers to be a polar body, is likewise absent; which is a further argument to be added to those already presented by me[1] for considering this cell a yolk cell rather than a polar body.

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[1] Young, R. T., "The Histogenesis of the Reproductive Organs of *Tænia pisiformis*," *Zool. Jahrb.*, XXXV., 355–418.

ASSOCIATION OF SOMATIC AND GERM CELLS IN CESTODES.

R. T. YOUNG.

The differentiated tissues of cestodes all arise from the parenchyma, primarily of the larva and secondarily of the adult. From this are formed the sex cells, there being no evidence whatever to indicate any specialized "germ path" or "germ cell determinants." One author[1] indeed has gone so far as to claim that specialized cells (muscle) may even be de- and redifferentiated to form germ cells.

How far these observations of Child are valid is open to question. His figures certainly give considerable support to his contention, but it is difficult to prove that any muscle directly contiguous to a developing testis is responsible for the origin of the latter, or that the testis represents its original myoblast. The contiguity may be accidental, and the parent muscle cell may lie at a different place and be separated from the muscle fibre by a considerable space, being connected to it by only a slender process. The occurrence of multinucleate myoblasts, some of which Child has figured and which I have occasionally found in my own slides, renders his interpretation reasonable, but by no means proves it.

The occurrence of fully differentiated flame cells within the testis of several species[2] of cestodes, unconnected with any other flame cells or excretory capillaries is suggestive, although not proof of a common origin of these cells. It is quite possible that the flame cells may have arisen outside of the testis and migrated into the latter secondarily, or the testis and flame cell may have developed side by side, the latter becoming surrounded by the offspring of the former.

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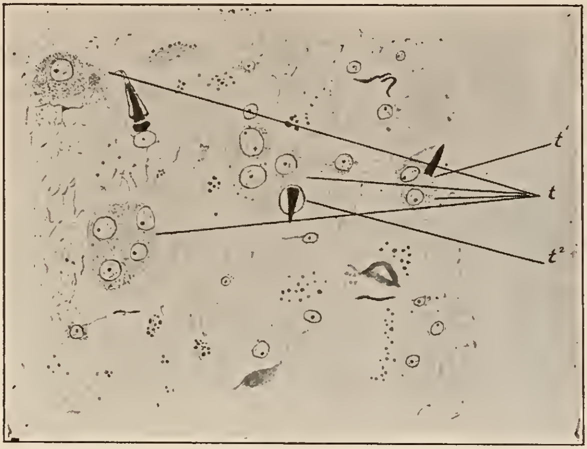
[1] Child, C. M., "Studies on the Relation between Amitosis and Mitosis, II., Development of the Testis and Spermatogenesis in *Moniezia*, BIOL. BULL., XII. 175–212.

[2] *Rhyncobothrium bulbifer*, *Dipylidium caninum* and an undetermined species from a woodpecker (*Dryobates*).

In the accompanying figure, however, I have shown what I consider good evidence in support of the former view.

This shows part of a proglottid of *Dipylidium caninum* containing numerous early testes. One of these contains but two cells, of which one is a developing flame cell (t') showing only the cone of cilia, while the other is a testis cell. Another shows several testis cells and one developed flame cell (t^2), which is not yet however connected with any capillary.



A part of the parenchyma of *Dipylidium caninum*, showing developing testes (t), two of which contain flame cells (t^1 and t^2), camera drawing. × 900.

Obviously, I cannot prove that the groups containing t^1 and t^2 are developing testes. Their similarity of structure to neighboring testes is however sufficiently evident.

But whether or not these flame cells and the cells of the testes in which they lie are of common parentage, their occurrence within the testes is evidence of the simplicity of cestode development; for it shows that apparently any cell of one tissue (testis or parenchyma) may develop into a specialized cell of another tissue (flame cell) entirely apart from any other cells of the latter; the apparently determining factor being a stimulus of the physiological environment.

The question why two cells in close proximity to each other and probably of common descent, should develop on the one hand into a flame cell, and on the other into a testis cell, cannot of course be answered in the present state of our knowledge. It is not unreasonable to suppose however that, as the result of some slight initial difference, one might become more susceptible to the presence of excreta in its environment and so develop into a flame cell; while the other, less susceptible to such influence, would follow its original course of development and become a testis cell.

There is in these cases evidently no continuity of development of the various parts of the excretory system as contended by Blochmann[1] and others, and discussed by me in an earlier paper.[2]

Such facts further clearly argue against the Roux-Weismann theory of mosaic inheritance, and in favor of Driesch's view that developing cells are totipotent, so far at least as cestodes are concerned.

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[1] Blochmann, F., "Die Epithelfrage bei Cestoden und Trematoden," Hamburg, 1896.

[2] Young, R. T., "The Histogenesis of *Cysticercus pisiformis*," Zoöl. Jahrb., XXVI., 183–254.

THE EXCRETORY SYSTEM IN DIGENEA. I.

ERNEST CARROLL FAUST.

Notes on the Excretory System of an Amphistome, *Cercaria convoluta*, nov. spec.[1]

Recently the excretory system of certain distomes has been described. Sewall Wright (1912: 167–170) made a thorough study of the system in the adult *Microphallus opacus*. Cort (1918, 1918a) and the writer (1918, 1918a) have added to the knowledge of these organs in cercariæ and distomula. However, no significant work on the excretory organs of amphistomes has been published since the important monograph of Looss (1892) on *Diplodiscus subclavatus* (Rudolphi). During the past year the writer has been enabled to make a detailed study of the cercaria and parthenita of an undescribed amphistome for which the name *Cercaria convoluta* is proposed. Although the main purpose of this paper is to elucidate the excretory system of this amphistomulum, a brief description of the other organs is made as a matter of record.

Cercaria convoluta nov. spec.

Host: *Planorbis trivolvis* Say.
Parthenita: redia.
Habitat: Urbana, Illinois.
Date: April to November, 1918.
Systematic position: Diplodiscinae.

Cercaria convoluta is a pyriform larva, measuring from 0.4 to 0.76 mm. in length by 0.3 to 0.56 mm. in width. The tail is approximately twice as long as the body. The oral cavity has a maximum outer diameter of 0.1 mm. The acetabulum, situated at the posterior margin of the body, has a width diameter of 0.16 mm. and a length diameter of 0.12 mm. It is directed ventrad. The pharyngeal pockets are large and conspicuous.

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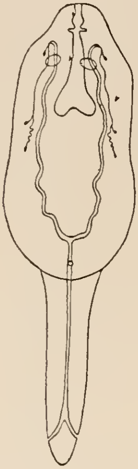
[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 130.

The esophagus is short; it is surrounded by a small bulbous pharynx just at the point where it opens into the diverticula. The latter extend caudad as far as the excretory pore and are usually undulatory in the adult larva. The genital organs are represented by four cell masses along the median line, suggesting ovary, testes and cirrus sac. Chords of cells run from the generative organs to the genital pore. Two large eye-spots are situated dorsad in the plane where the divericula originate. They are composed of oval "lenses" surrounded by a mass of melanoidin granules. Dense patches of granules entirely obscure this region. Cystogenous granules form dense aggregates in the connective tissue of the worm. On placing the fluke in water they are freely thrown out and soon dissolve to form a mucus cyst capsule. Meanwhile the tail is easily loosed from the body and cast aside. This species probably belongs to the subfamily Diplodiscinæ.

Cercaria convoluta develops within a redia with conspicuous "feet" and a large irregular pouch-like gut. The pharynx at the anterior end of the redia is small. An inconspicuous birth-pore is present. The redia may reach a length of 1.5 to 2 mm.

While the above description suffices to separate *Cercaria convoluta* from previously described cercariæ, this species is most strikingly delimited by the convolutions of the main excretory tubules of the cercaria. The excretory pore is located just in front of the anterior margin of the acetabulum. It is surrounded by a strong sphincter, and opens into a small bladder. From the latter organ a single median longitudinal canal runs caudad for about two-thirds the length of the tail. It then bifurcates, each fork opening to the exterior in the distal fifth of the tail. Immediately in front of the bladder is a horizontal collecting tubule which receives the excretory products from right and left sides. As this tubule reaches the sides of the acetabulum it is directed forward. In the very young cercaria it has a serpentine course. This tendency becomes progressively more pronounced as the larva develops, until in the mature cercaria it consists of a spiral of about six definite loops. At the place where the lateral collecting tubule reaches the eye-spot it reflexes outward and backward. Its course may be traced to the region of the bladder where it runs into the acetabulum. The posterior limit of the main collecting tubule of each side is marked by a bifurcation. The outer (posterior) fork remains free, but the inner (anterior) one joins its mate of the opposite side and thus secures a physiologically important connection between the excretory courses of the two sides of the body.

The flame cells have been carefully studied in living flukes. In the mature cercaria they are naturally divided into three groups. At the anterior end, given off just after the collecting tubule flexes caudad, there is a cluster of eight cells on each side of the body. Midway down on the reflexed portion of the tubule a single large flame cell is found. In the acetabulum the outer (posterior) tubule of the posteriormost group terminates in a cluster of eight cells. The inner (anterior) likewise gives rise to a cluster of eight terminal flame cells. Analysis shows that the cephalic group of eight cells and the two octet groups in the acetabulum all have an orderly arrangement and are the result of a regular development. Each consists of a quadruple bifurcation of an originally single flame cell. Moreover, this analysis is borne out by the successive stages in the development of the system. The collecting system is originally composed



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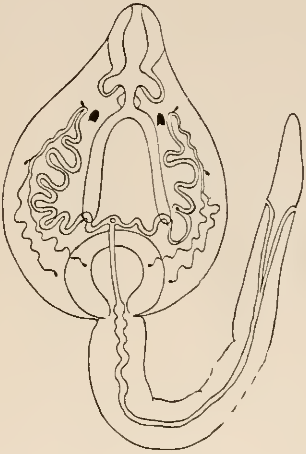

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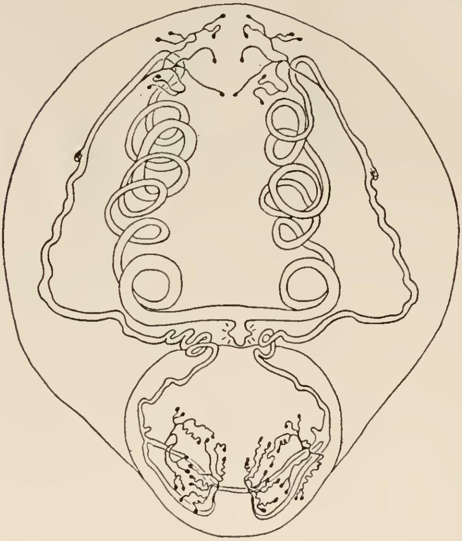
FIG. 1. Very young *Cercaria convoluta*, showing three flame cells on each side of the body. × 250.

FIG. 2. Four cell stage in development of excretory system of *C. convoluta*. × 225.

of a single pair of tubules. Fusion of these two canals occurs at an early stage in the region of the bladder and anterior part of the tail. When the pigmentation of the eye-spots first becomes conspicuous three groups of flame cells are already present, each group being represented by a single cell (Fig. 1). Later, when the animal assumes a more characteristic shape (Fig. 2), the posteriormost cell and tip of the tubule has bifurcated. With the maturing of the cercaria successive stages of division of the anteriormost flame cell into two, four and eight cells may be found. The middle cell remains single, which fact accounts for its disproportionately large size. In the posteriormost group the main canal of the inner (anterior) tubule approaches its mate of the opposite side and fuses with it. The cell becomes oriented laterally and gives rise to eight cells. In like manner the outer (posterior) tubule produces eight flame cells near its median margin. Thus there are formed (Fig. 3) twenty-five flame cells on each side of the body of the mature *Cercaria convoluta* from three original groups of one flame cell each.



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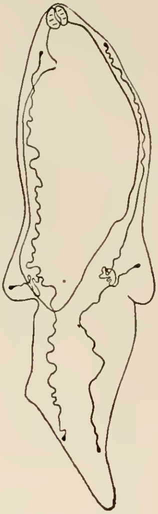

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Fig. 3. Mature *Cercaria convoluta*, with 25 flame cells on each side of the body. × 175.

Fig. 4. Excretory system in the parthenita of *C. convoluta*. × 175.

Proof of the fundamental character of the groups and numbers of flame cells in *Cercaria convoluta* is further established by the analysis of the excretory system of the redia. Here (Fig. 4) there are three groups of flame cells on each side of the body, although the more primitive character of the redia is evidenced by the single cell as representative of each group.

Discussion.

The exact knowledge of the groups of flame cells and exact grouping of these cells in *Cercaria convoluta* makes it possible to understand the importance of the excretory system both in the anatomy and systematology of the group. In his study of *Diplodiscus subclavatus* Looss has shown that the miracidium of his species has a single pair of flame cells at the anterior end of the unbranched collecting tubules. With the metamorphosis into a sporocyst the collecting tubules elongate but the single flame cell on each side persists. In the redia of this species Looss has found from two to four flame cells on each side of the median line, although he is not sure if the number is constant. In the writer's species there are always three flame cells on each side of the redia. In the cercaria germ-ball in both *C. diplodisci subclavati* and *C. convoluta* there is at first a single flame cell for each lateral collecting tubule. The next stage recognized by Looss in his species is one with four flame cells, each of which appears to represent a separate group. A subsequent stage in Looss's figures shows three cells for the cephalic group, two each for the two groups in the middle of the body and eight (or nine) for the acetabular group. This same number persists in the

Table I.

Showing Number of Flame Cells Respectively in Cercaria convoluta and C. diplodisci subclavati at various stages in the Life History.

	Miracidium.	Sporocyst.	Redia.	Cercaria.				Mature Worm.
				Stage 1.	Stage 2.	Stage 3.	Stage 4.	
C. convoluta . .	?	?	3	1	1+1+1	1+1+1+1	8+1+8+8	?
C. diplodisci subclavati. . .	1	1	4 (?)	1	1+1+1+1	omitted	3+2+2+8 (9)	?

mature cercaria. In *Cercaria convoluta*, on the other hand, a distinct three-cell stage is passed through. The four-cell stage is found only after the acetabular cell has divided. Then by successive divisions eight cells are formed from the cephalic and each of the acetabular groups, while the cell in the mid region of the body fails to divide. Thus in the mature cercaria of *Diplodiscus subclavatus* there are fifteen or sixteen flame cells on each side of the body while in the writer's species twenty five flame cells have been consistently found.

In spite of the less pronounced symmetry of division of flame cells in *Cercaria diplodisci subclavati* it is nevertheless highly probable that the grouping is of a fourfold character both in redia and cercaria, just as the arrangement in *C. convoluta* is threefold. Thus within a relatively small subfamily there exists a fundamental difference of arrangement of a relatively conservative system.

SUMMARY.

1. *Cercaria convoluta*, a new species belonging to the Diplodiscinæ, is described.

2. The development of flame cells in both redia and cercaria of this species is based on a three-fold division of a primitively single flame cell on each side of the body.

3. A study of the development of flame cells in *C. diplodisci subclavati*, based on Looss's study, shows a four-fold group arrangement but with less exact symmetry.

4. A comparison of these two forms shows a fundamental difference of arrangement of a relatively conservative system within the same subfamily.

REFERENCES CITED.

Cort, W. W.
'18 Homologies of the Excretory System of the Forked-Tailed Cercariae. Jour. Parasit., 4, 49–57.
'18a The Excretory System of *Agamodistomum Marcianae* (La Rue), the Agamodistome Stage of a Forked-Tailed Cercaria. Jour. Parasit., 4, 130–134.
Faust, E. C.
'18 Life History Studies on Montana Trematodes. Ill. Biol. Monogr., 4, 1–120, 9 pl.
'18a Studies on Illinois Cercariae. Jour. Parasit., 4, 93–110, 2 pl.

Looss, A.

'92 Recherches, sur la Faune Parasitaire de l'Égypte, prem. partie. Mem. Inst. Égypt., 3, 1–252, 15 pl.

Wright, Sewall

'12 Notes on the Anatomy of the Trematode, Microphallus Opacus. Trans. Am. Micr. Soc., 31, 167–175, 2 pl.

THE EXCRETORY SYSTEM IN DIGENEA. II.

ERNEST CARROLL FAUST.

Observations on the Excretory System in Distome Cercariæ.[1]

The diverse groups connoted by the term "distome cercariae" are found to be decidedly heterogeneous when the reproductive and excretory systems are examined. Even the nervous system is extraordinarily modified in one family of this group, the Schistosomatidæ. Students of distome cercariae have commonly made mention of the bladder and have frequently observed the main collecting tubules of the excretory system. Only infrequently, however, have they traced out accurately the auxiliary tubules and made an exact analysis of the flame cells at the distal ends of the capillaries. Even as accurate an observer as Looss has often been unable to analyze the system in material which was available for study as living mounts. Failure to determine the number of flame cells and their exact relationship to the collecting tubules may be due to (1) paucity of material at hand, (2) concealment of the cells by cystogenous or mucin glands or heavy and spinose integument, or (3) insufficient time to make careful observations at the exact moment when the material is best fitted for study. In general, mature free-swimming cercariæ are much the best for this study and freshly dissected material is much better to work on than that which has been standing for several hours. It is true, however, that material may present seemingly insuperable difficulties for the study of the excretory system at a certain time or when taken from a certain place, while at another time or in another locality the study of the same species may be one of comparative ease.

Cort has frequently noted that the study of the excretory system is both tedious and wearisome. The writer may add that a successful analysis of the system requires greater care and more poise of judgment than that of any other system of the fluke.

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[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 131.

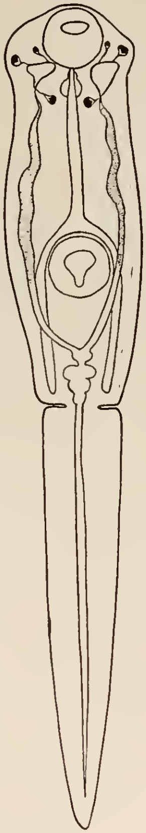
It is the purpose of this paper to present data on the excretory system of three distinct groups of distome cercariæ, the echinostome cercariæ, the xiphidiocercariæ and the furco-cercariæ, and to show how these data may be used to supplement the anatomical relationships based on other fundamental systems of these platodes.

ECHINOSTOME CERCARIÆ.

These cercariæ, frequently designated as the offspring of rediæ, with a collar of spines at the anterior end of the body, may be as readily recognized by the pair of heavy collecting tubules extending from the bladder to the region of the pharynx, where they become constricted and reflex on themselves in triangular fashion (see Figs. 1 and 2). The manner in which the flame cells are disposed, together with their number, and the course of the tubule in the tail offer a method for separating larval echinostomes into two quite distinct groups.

The more simple of these groups, as illustrated in Fig. 1, shows the collecting tubule ending in the triangular flexure and only three flame cells for each half of the body. The cells are situated at the ends of small capillaries which empty, the one at the end, the others at the sides of the triangle. No other flame cells occur along the entire course of the tubules. In the tail a single median tubule runs from the distal end forward into the bladder. Of the cercariæ in which the flame-cell count has been satisfactorily worked out, two species fall into this group, *Cercaria chisolenata* Faust, 1918, and *C. trisolenata* Faust, 1917.

The second or more complex type is one in which the constricted tubule does not end in the region of the triangular flexure lateral to the pharynx but continues caudad to the posterior region of the body. Commonly (Fig. 2), when this secondary portion of the tubule reaches the posterior region of the body, it bends forward and continues to the region of the pharynx. Numerous flame cells, constant for each species, are connected by minute capillaries with this tertiary portion of the tubule. In *Cercaria acanthostoma* Faust, 1918, sixteen flame cells occur along each tubule. In *Cercaria complexa* nov. spec. the number is fifteen. In this type also the main collecting tubule of the tail, after coursing distad for a short distance, bifurcates and opens on each side through a simple pore. To this type belong *C. trivolvis* Cort and *C. reflexae* Cort in which the flame cells have not been worked out. The cercaria of *Echinostomum revolutum* (Froel.) is also a member of this group but is not entirely typical, for in this species the tertiary portion of the collecting tubule arises as a bifurcating branch from the mid-region of the secondary portion of the canal. Flame cells occur along both rami of the tertiary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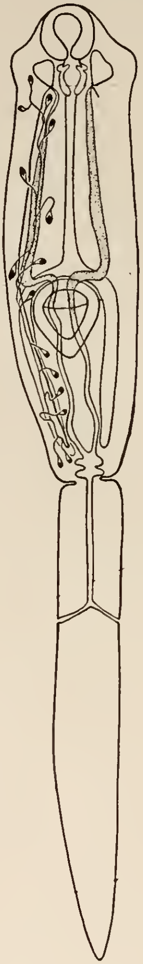
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FIG. 1. *Cercaria chisolenata* Faust, ventral view, showing excretory and digestive systems. × 105.

FIG. 2. *Cercaria complexa* nov. spec., ventral view, showing excretory and digestive systems. × 170.

tubule and probably also along the posterior part of the secondary tubule. The exact number of flame cells for each side of the body is not clearly shown but somewhere between eighteen and twenty may be counted (Looss, 1894: Fig. 191).

A species showing relationships intermediate between these two groups is *Cercaria biflexa* Faust, 1917. Both secondary and tertiary portions of the tubule occur here (Faust, 1918, Fig. 138), but they are confined entirely to the head region of the body. Numbers of tributaries to the primary tubule (Faust, 1918, Fig. 135) empty into that canal but no flame cells have been found in connection with them. In the tail of this species the main collecting tubule forks about two-fifths the way distad. The forks continue to the end of the tail but have not been found to end in pores to the exterior. Numerous branches empty into the single and bifurcated portions of the canal.

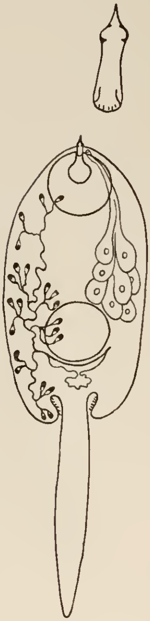
In the matter of origin the species with three flame cells in the anterior and of the worm are undoubtedly the simpler. It is not improbable that the three anteriormost flame cells of the more complex type are homologous to the three cells of the simpler type. This theory is supported by the evidence from *C. biflexa,* which, with the flexures similar to *C. complexa,* has the flame cells of the anteriormost portion of the tertiary tubule.

In view of the relationship existing among these species it seems feasible to classify them in groups on the basis of a *flame-cell formula.* Thus the flame-cell formula of *Cercaria chisolenata, C. trisolenata* and *C. biflexa* is 3, that of *C. complexa* is 15 = (3 + 12), and that of *C. acanthostoma* is 16 = (3 + 13). Further study of the flame cells in larval echinostomes will probably furnish evidence of other types and many other points of fundamental interest.

XIPHIDIOCERCARIÆ.

Anatomically the xiphidiocercariæ have little in common. Each species quite probably has a distinct type of stylet but no satisfactory classification is likely to result on the basis of this larval piercing organ. Likewise the genital and excretory systems show very great differentiation. The writer (1918, 38–40) has shown and described some of the fundamental types of bladder and main collecting tubules of this group. Ordinarily the collecting tubules arrange themselves in anterior and posterior branches.

The first xiphidiocercaria in which the writer was able to make an analysis of the number and relation of flame cells was *Cercaria isocotylea* Cort (Faust, 1918a; also Fig. 3, this paper).



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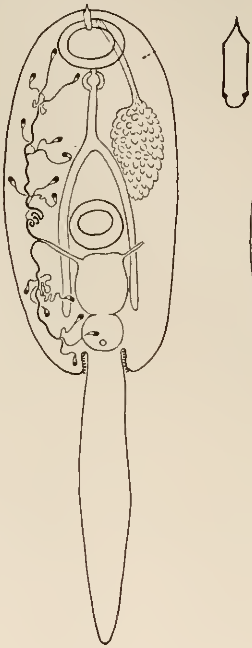

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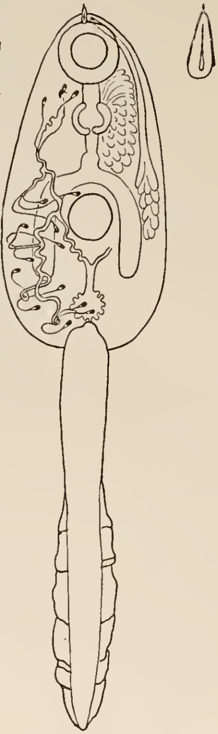

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FIG. 3. *Cercaria isocotylea* Cort, ventral view, showing excretory system and mucin glands. × 170. 3*a*, stylet, × 540.

FIG. 4. *Cercaria candelabra* nov. spec., ventral view, showing excretory and digestive systems. × 170. 4*a*, stylet, × 540.

FIG. 5. *Cercaria trifurcata* nov. spec., ventral view, showing excretory and digestive systems. × 105. 5*a*, stylet, × 540.

The number of flame cells is large for a cercaria and the arrangement is complex. Placing the cells of this species into anterior and posterior groups respectively, the flame-cell formula is

$$22 = ([2 + 2 + 3 + 3 + 3 + 3] + [2 + 2 + 2]).$$

More recently the writer has studied the excretory system in two xiphidiocercariæ, also from Urbana, Illinois, *C. trifurcata* nov. spec., and *C. candelabra* nov. spec., both with an equal number of flame cells and with the same number to the group, but with a different disposition of the groups anteriad and posteriad. Thus the flame-cell formula of *C. trifurcata* is 15 = ([3 + 3] + [3 + 3 + 3]), while that of *C. candelabra* is 15 = ([3 + 3 + 3] + [3 + 3]).

Although these three species constitute the only known xiphidio-cercariæ in which the flame-cell number and grouping have been worked out to a certainty, very considerable light is thrown on the subject by a comparison of these larval species with related adult species. *Allocreadium isoporum* (Looss, 1894, Fig. 103) has a flame-cell formula of 24 = ([4 + 4 + 4 + 4] + [4 + 4]). The larva is a stylet cercaria with an unusually large tail. The larva of *Acrolichanus petalosa*, belonging to the same family, has the same fundamental flame-cell grouping but reversed as regards number of units disposed anteriad and posteriad. The formula is 12 = ([2 + 2] + [2 + 2 + 2 + 2]). *Anchitrema sanguineum* (Looss, 1896, Fig. 77) has a flame-cell formula of the same number of groups but different disposition of the groups and different numbers of cells within the groups, *i. e.*, 16 = ([2 + 3 + 3] + [3 + 3 + 2]).

Two plagiorchiine species, *Haplometra cylindracea* and *Opistoglyphe endolobum* (Looss, 1894, Figs. 29, 163) have larvae which are typically xiphidiocercariæ. Both of these adult species have the same flame-cell formula, 18 = ([3 + 3 + 3] + [3 + 3 + 3]). The very young distomulum of *Opistoglyphe* has a formula of 6 = ([1 + 1 + 1] + [1 + 1 + 1]), from which it is plainly to be seen that the adult condition is derived by a triad splitting of each fundamental group. That species in two different subfamilies of the Plagiorchiidæ should have identical flame-cell formulæ is quite significant.

Again, certain subfamilies of the Brachycœliidæ, in which the larvae are thought to be xiphidiocercariæ, have a flame-cell formula of 12 = ([3 + 3] + [3 + 3]), while *Microphallus opacus* (Wright, 1912), belonging to another subfamily of the Brachycœliidæ, has a formula of 8 = ([2 + 2] + [2 + 2]). Analysis shows that these two species have a *common larval denominator*, namely 4 = ([1 + 1] + [1 + 1]). In other words, the mathematical exactness of flame-cell formation in this family makes it possible to calculate the flame-cell formula of the cercaria from the condition of the adult.

The study of the flame-cell structure in the xiphidiocercariæ and comparison of the system with that of the related adult flukes show in the first place that the fundamental basis of the system is the number and disposition of the flame-cell groups. Knowledge of the structure of the excretory system in the Plagiorchiidæ and Brachycœliidæ makes it possible to state that this fundament in these groups is a family or subfamily character. The number of flame cells in each basic group is of lesser importance. Thus in the plagiorchiine species, where the species is known, triad groups are the rule, but in the brachycœliine species diad formation occurs in one subfamily and triad formation in another. In the second place this study has caused the writer to believe that this system is decidedly conservative in the xiphidiocercariæ.

A study of *Cercaria trifurcata* and *C. candelabra* shows these species to have a flame-cell formula intermediate in form between that of the Brachycœliidæ and the Plagiorchiidæ. *C. trifurcata* represents a type in which the anterior fundament is brachycœliine and the posterior fundament plagiorchiine, while *C. candelabra* represents just the reversed condition. On account of the large number of flame cells already present in the larva it is highly probable that both of these species represent a condition of flame-cell development which for the cercaria is somewhat more precocious than that of the larvæ of the Brachycœliidæ and the Plagiorchiidæ. The system in *C. isocotylea* has reached a high degree of complexity which bears homologous relationships to no other known system in larval or adult flukes.

FURCOCERCARIÆ.

The forked-tailed cercariæ have secured more than ordinary attention during the past few years because experimental evidence has shown some of them to have a genetic relationship to the three human blood flukes, *Schistosoma hæmatobium*, *S. mansoni* and *S. japonicum*. Cort (1918)has made a careful analysis of the excretory systems in five furcocercariæ and the writer (1918a) has previously published flame-cell data for the species *C. gigas*. The writer's study of four additional species tends both to confirm Cort's data and, in supplementing and expanding these data, to emphasize the writer's previous view (1918a, 108) that it seems "reasonable to recognize a complete series of larva forms from those with a pharynx sphincter . . . to the human schistosome cercariæ."

Of the ten species of forked-tailed cercariæ for which adequate data are at hand with respect to the flame-cell structure, the cercaria of *Schistosoma japonicum* presents the most simple basic plan. Following the method previously used in this paper, the flame-cell formula of this species is $4 = (2 + 2)$. That one of these flame cells in each lateral circuit is found in the caudal portion of the larva is incidental rather than fundamental. The species *Cercaria douthitti* and *C. elephantis* of Cort represent

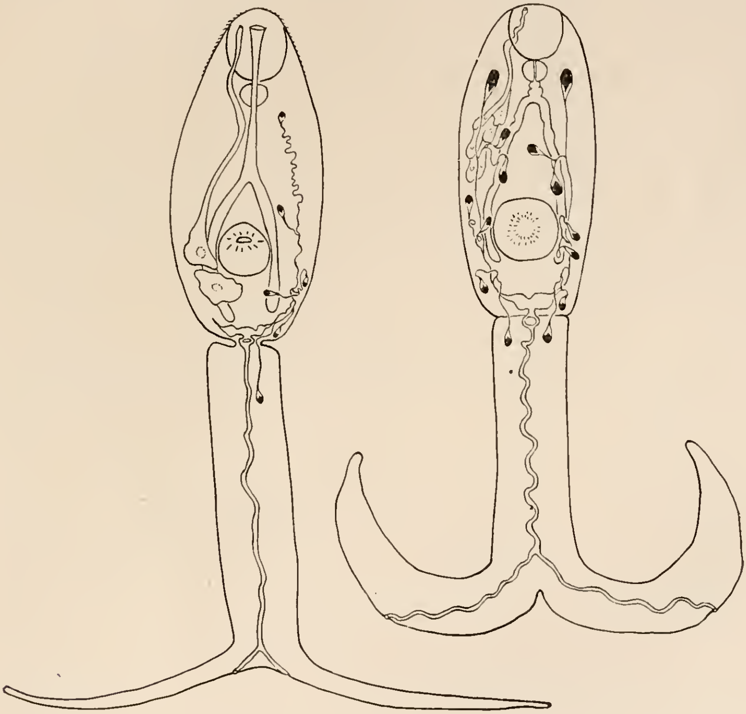

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FIG. 6. *Cercaria furcicauda* nov. spec., ventral view, showing excretory and digestive systems. × 170.

FIG. 7. *Cercaria robusticauda* nov. spec., ventral view, showing excretory and digestive systems. × 540.

a type with a slightly more complex formula, $6 = (3 + 3)$. The fundament is, however, just the same, $2 = (1 + 1)$, so that these two may be considered two subdivisions of one larger group, all members of which have the same basic formula.

A simple condition of a stage in advance of this primitive group cited above is expressed in the excretory system of *Cercaria furcicauda* nov. spec. (Fig. 6), in which there are three instead of two basic groups. The formula for the flame cells in this species is $6 = (2 + 2 + 2)$. One of these flame cells is in the tail. Two species, evidently differing in minor characters but bound together by the same number and arrangement of flame cells are *C. emarginatæ* and *C. douglasi*. These differ from

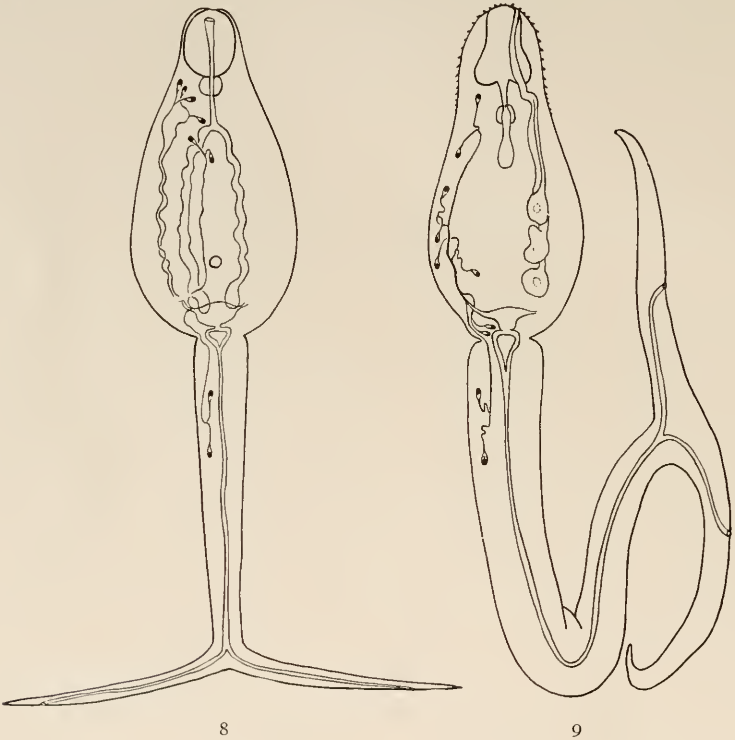

FIG. 8. *Cercaria quattuor-solenata* nov. spec., ventral view, showing excretory and digestive systems. × 105.

FIG. 9. *Cercaria rhabdocaeca* nov. spec., ventral view, showing excretory and digestive systems. × 330.

C. furcicauda in having three flame cells instead of two in the first (anterior) group. The formula for these species is 7 = (3 + 2 + 2). In *Cercaria robusticauda* (Fig. 7) there are the same number of flame cells as in *C. emarginatæ* and *C. douglasi*, but the size of the anteriormost is evident proof of its double nature. Hence the formula for this species is 7 = (1 + 2 + 2 + 2). One of these cells runs into the tail. This species is transitional between the larvæ with seven flame cells in three groups and *C. quattuor-solenata* nov. spec.

C. quattuor-solenata (Fig. 8) represents a condition in which a full additional flame-cell unit has been provided. Its formula is 8 = (2 + 2 + 2 + 2). Incidentally, the main collecting tubule in this species has been shortened to a minimum, while the four secondary tubules have been lengthened accordingly.

In *Cercaria rhabdocæca* nov. spec. (Fig. 9) five fundamental flame-cell groups are found, so that the formula is 10 = (2 + 2 + 2 + 2 + 2).

The flame-cell arrangement in *Cercaria gigas* (Fig. 10) is apparently unrelated to any of the foregoing species. Ten flame cells have been found on each side of the body of the cercaria and one in the tail. By inspection the formula would seem to be 10 = (2 + 1 + 1 + 1 + 1 + 3 + 1). That this may constitute a seven-fold grouping, in which two of the groups have divided, is supported in part by the fact that the single cells are somewhat larger than the cells of the units where the division has taken place, but the evidence is not entirely convincing. In the mature cercaria of this species the flame cell in the tail has no direct connection with the cells or tubules of the body proper. Its outlet is through a simple longitudinal

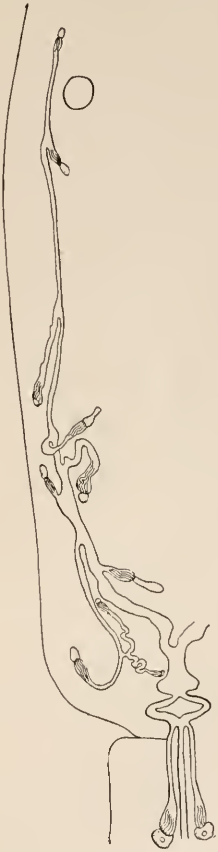

FIG. 10. *Cercaria gigas* Faust, dorsal view, showing excretory system on right side of body. × 540.

vessel which empties into the bladder at the side of the median collecting tubule of the tail, just behind *the island of Cort.*

In all of these furcocercariæ, as well as in others where the flame cells have not been worked out, there is a forking of the caudal excretory tubule at the junction of furci with tail trunk. The forked tubules empty through simple pores at the sides or distal extremity of the tail-furci. Comparison of the point of opening of these pores in various species shows that in the cercaria of *Schistosoma japonicum*, *C. douthitti* and *C. elephantis* the openings are at the tips of the furci; that in most other forms they are half-way out on the sides; but that in *C. furcicauda* the outlets are near the origin of the furci (Fig. 6). Thus the writer sees no consistent correlation between the flame-cell formulæ and the point of outlet of the caudal tubules.

In furcocercariæ the excretory system is apparently always provided with *the island of Cort.*

Analysis of the flame-cell fundaments in this group shows a simple basic plan and an unusually complete series of stages from the simplest combination of units (cercaria of *S. japonicum*)

TABLE I.

METHODS FOR DISTINGUISHING THE BETTER KNOWN FORKED-TAILED CERCARIÆ.

Species.	Flame-cell Formula.	Digestive Tract.	Eye-spots.
Cercaria of *Schistosoma japonicum*	$4 = (2 + 2)$	pharynx absent, 5 pairs mucin glands.	absent.
Cercaria douthitti	$6 = (3 + 3)$	pharynx absent, 5 pairs mucin glands.	present.
Cercaria elephantis	$6 = (3 + 3)$	pharynx absent, 25–30 pairs mucin glands.	present.
Cercaria furcicauda	$6 = (2 + 2 + 2)$	pharynx present, 2 pairs mucin glands.	absent.
Cercaria emarginatæ	$7 = (3 + 2 + 2)$	pharynx present, mucin glands?	absent.
Cercaria douglasi	$7 = (3 + 2 + 2)$	pharynx present, 2 pairs mucin glands.	absent.
Cercaria robusticauda	$7 = (1 + 2 + 2 + 2)$	pharynx glandular, 6 pairs mucin glands.	absent.
Cercaria quattuor-solenata	$8 = (2 + 2 + 2 + 2)$	pharynx present, no mucin glands.	absent
Cercaria rhabdocæca	$10 = (2 + 2 + 2 + 2 + 2)$	pharynx present, rhabdocœl gut, 3 pairs mucin glands.	absent.
Cercaria gigas	$10 = (2 + 1 + 1 + 1 + 1 + 3 + 1)$ (?)	pharynx absent, mucin glands many, of two kinds.	present.

to a rather large aggregate of such units (*C. rhabdocæca*). *C. gigas* is apparently not related to this series, since an analysis of its flame cells fails to show any direct correlation with the established series.

Discussion.

The data presented in this paper are of fundamental significance in establishing the relationships of various larval flukes to one another and of larvæ to adults. Present knowledge not only preponderates in favor of the view that the number of flame cells in the species is constant, but establishes the belief also that the same basic number and arrangement of flame cells exist within families or subfamilies. When once the number and disposition of flame cells has been established for a particular group it will be possible to predict the flame-cell formula of the larvæ. Thus a larva to belong to the Schistosomatidæ *sensu stricto* should possess a flame-cell arrangement consistent with the *common denominator* which the apharyngeal species of furcocercariæ possess in common, namely $2 = (1 + 1)$; and a plagiorchiine species should have the common formula of that family, $6 = ([1 + 1 + 1] + [1 + 1 + 1])$; and brachycœliine species should follow the scheme $4 = ([1 + 1] + [1 + 1])$. This accords with Cort's thesis on the conservatism of the excretory system, but it suggests, from the number of types already known, a larger number of possibilities of flame-cell arrangement than had been previously anticipated, and, accordingly, a larger number of family groups than the present knowledge of adult flukes postulates.

Because the number of basic groups is the most significant point in the analysis of the flame-cell arrangement of cercariæ and adults alike, there is justification for presenting general formulæ of flame-cell units which will apply equally well to all species of the same group. These formulæ are readily obtained if the *common denominator* of the species of the same group is taken and literal equivalents substituted for the numbers involved. Thus for the species of Schistosomatidæ the formula would read $\alpha + \beta$; for Brachycœliidæ $\alpha' + \beta' + \alpha'' + \beta''$, where the primed letters represent the anterior groups and the double-primed letters represent the posterior groups. Under this scheme the echinostome larvæ with three flame cells on a side are given the formula of α, while those with other flame cells distributed along the tertiary collecting tubule behind the α group would be designated as $\alpha + \beta^n$. The full scheme for litteral formulæ for the larval and adult flukes where the flame cells have been best analyzed is presented in the following table (Table II.).

TABLE II.

GENERAL FORMULÆ FOR FLAME-CELL ARRANGEMENT IN DISTOMATA.

ECHINOSTOMES.	
Cercaria chisolenata	α
Cercaria trisolenata	
Cercaria biflexa	
Cercaria complexa	$\alpha + \beta^n$
Cercaria acanthostoma	
XIPHIDIOCERCARIÆ.	
Cercaria trifurcata	$\alpha' + \beta' + \alpha'' + \beta'' + \gamma''$
Cercaria candelabra	$\alpha' + \beta' + \gamma' + \alpha'' + \beta''$
Cercaria isocotylea	$\alpha' + \beta' + \gamma' + \vartheta' + \epsilon' + \xi' + \alpha'' + \beta'' + \gamma''$
BRACHYCŒLIIDÆ	$\alpha' + \beta' + \alpha'' + \beta''$
PLAGIORCHIIDÆ	$\alpha' + \beta' + \gamma' + \alpha'' + \beta'' + \gamma''$
ALLOCREADIIDÆ.	
Allocreadium isoporum	$\alpha' + \beta' + \gamma' + \vartheta' + \alpha'' + \beta''$
Acrolichanus petalosa	$\alpha' + \beta' + \alpha'' + \beta'' + \gamma'' + \varphi''$
FURCOCERCARIÆ.	
Cercaria of Schistosoma japonicum	$\alpha + \beta$
Cercaria douthitti	
Cercaria elephantis	
Cercaria furcicauda	$\alpha + \beta + \gamma$
Cercaria emarginatæ	
Cercaria douglasi	
Cercaria robusticauda	$\alpha + \beta + \gamma + \vartheta$
Cercaria quattuor-solenata	
Cercaria rhabdocœca	$\alpha + \beta + \gamma + \vartheta + \epsilon$

DESCRIPTION OF NEW SPECIES CITED IN THIS PAPER.

Cercaria complexa nov. spec. (Fig. 2.)

Systematic position: echinostome larva.

Parthenita: redia.

Host: *Planorbis trivolvis* Say.

Habitat and time of collection: drainage ditch, Urbana, Ill., 1918.

Flame-cell formula: $\alpha + \beta^n = 3 + 12$ or 15.

Body 0.36 mm. long by 0.10 mm. wide; tail 0.36 mm. long by 35 μ in diameter at base. Oral sucker 35 μ in diameter, acetabulum 50 μ in diameter. Entire ventral surface of body between pharynx and acetabulum sunken into elongate bowl-like sucking organ. Collar spines 38, disposed in two alternating rows.

Redia with conspicuous "feet," rhabdocœl gut, collar prominence and birthpore.

Prepharynx short; pharynx urn-shaped; esophagus long, capillary; furci distended, extending to posterior tip of body. Mucin glands six, with ducts tipped with piercing spines.

Excretory tubules with double flexure; 15 flame cells along the entire length of the tertiary portion.

Cercaria trifurcata nov. spec. (Fig. 5.)

Systematic position: stylet larva.

Parthenita: sporocyst.

Host: *Physa gyrina* Say.

Habitat and time of collection: drainage ditch, Urbana, Ill., 1918.

Flame-cell formula: $\alpha' + \beta' + \alpha'' + \beta'' + \gamma'' = 3 + 3 + 3 + 3 + 3$ or 15.

Body 0.43 mm. long by 0.2 wide in region of acetabulum; tail 0.43 mm. long by 50 μ in diameter at base, fluted laterally in distal half. Entire body covered with minute spines.

Sporocyst consisting of elongate compound branches.

Acetabulum 60 μ in diameter; weak and ineffective; oral sucker 76 μ in diameter, directed anteriad. Prepharynx provided with great mass of secretory glands; pharynx and esophagus present but inconspicuous; ceca broad sacculate pouches extending some distance behind the acetabulum; mucin glands several, in vitiform cluster, with granular contents, emptying through long ducts at sides of stylet.

Bladder small, crenate; main collecting tubule Y-form; anterior collecting tubule of each half of body with two triplet groups of flame cells and posterior collecting tubule with three triplet groups of flame cells.

Cercaria candelabra nov. spec. (Fig. 4.)

Systematic position: stylet larva.

Parthenita: sporocyst.

Host: *Planorbis trivolvis* Say.

Habitat and time of collection: drainage ditch, Urbana, Ill., 1918.

Flame-cell formula: $\alpha' + \beta' + \gamma' + \alpha'' + \beta'' = 3 + 3 + 3 + 3 + 3$ or 15.

Body 0.25 mm. long by 0.09 mm. wide; tail two-thirds to three-fourths of body length. Integument heavy; body entirely covered with spines. Oral and ventral suckers 44 μ in diameter. Stylet in roof of oral sucker 25 μ long. Tail inserted into caudal pocket with a lateral pair of about ten heavy spines each.

Sporocyst simple, unbranched.

Prepharynx short; pharynx simple; glands crowding parenchyma emptying into pharynx; esophagus moderately long; ceca long, small, to subcaudal region of body. Mucin glands in vitiform clusters, antero-laterad to ceca, opening through long narrow ducts at sides of stylet.

Excretory bladder consisting of proximal and distal vesicles separated from one another by slightly muscular sphincter; collecting tubule emerging from each side of anterior vesicle with anterior tributaries from three triplet groups of flame cells and with posterior tributaries from two triad groups of flame cells.

Cercaria furcicauda nov. spec. (Fig. 6.)

Systematic position: furcocercous larva.

Parthenita: sporocyst.

Host: *Anculosa carinata* Brug.

Habitat and time of collection: Rome, Georgia, 1918.

Flame-cell formula: $\alpha + \beta + \gamma = 2 + 2 + 2$ or 6.

Forked-tailed cercaria with body 0.26 mm. long and 0.12 mm. wide; tail trunk equally long; furci two-thirds length of tail trunk, extending at right angles to trunk. Anterior region of body covered with minute spines. Oral sucker 48 μ in diameter, directed anteriad; acetabulum 40 u in diameter, at beginning of posterior third of body. Eye-spots wanting.

Sporocyst simple, sausage-shaped sac.

Prepharynx and pharynx simple; esophagus moderately long; ceca typically furculate. Mucin glands two pairs, opening through long ducts at sides of oral atrium.

Excretory bladder small; collecting tubules swollen at base; three pairs of flame cells with capillaries opening into each collecting tubule, one flame cell of posterior couplet running back into tail.

Mass of germ cells just anterior to bladder.

Cercaria robusticauda nov. spec. (Fig. 7.)

Systematic position: furcocercous larva.
Parthenita: sporocyst.
Host: *Physa gyrina* Say.
Habitat and time of collection: drainage ditch, Urbana, Ill., 1918.
Flame-cell formula: $\alpha + \beta + \gamma + \vartheta = 1 + 2 + 2 + 2$ or 7.

Minute forked-tailed cercaria with body 75 μ in length by 25 μ in width, tail trunk equally long, but with short, stubby furci. Oral sucker 12 μ in diameter, directed forward; ventral sucker equal in diameter, somewhat back of middle of body, with two concentric rings of spines. Blunt spines covering anterior third of body. Eye-spots wanting. Morphological pharynx degenerate, with glandular instead of muscular cells; esophagus leading into furci which meander to posterior margin of acetabulum. Mucin glands about six on each side, homogeneous in character, opening through single ducts at each side of the oral aperture.

Excretory bladder bicornuate, with single collecting tubule running into cornu on each side. Flame cells based on four-unit plan, with a single cell in the anteriormost unit and a pair in each of the succeeding three.

Germ cells, consisting of a loose aggregate, in front of the bladder.

Cercaria quattuor-solenata nov. spec. (Fig. 8.)

Systematic position: furcocercous larva.
Parthenita: sporocyst.

Host: *Anculosa carinata* Brug.

Habitat and time of collection: Rome, Georgia, 1918.

Flame-cell formula: $\alpha + \beta + \gamma + \vartheta = 2 + 2 + 2 + 2$ or 8.

Forked-tailed cercaria with body 0.4 mm. long by 0.19 mm. wide, with tail trunk equally long and with furci about 0.26 mm. long, terminating in very short spinose porcesses. Eye-spots wanting.

Acetabulum 20 μ in diameter, in posterior fourth of body; oral sucker 70 μ in diameter, opening anteriad. Pharynx 30 μ in trans-section; esophagus of narrow bore, opening into thin glandular-walled ceca which run caudad as far as the bladder. Mucin glands wanting.

Sporocyst simple, sausage-shaped sac.

Excretory bladder oval, depressed; collecting tubules four to each side, arising simultaneously from the bladder. Flame cells in four paired groups, the posterior pair being in the tail.

Large mass of germ cells midway between acetabulum and bladder.

Cercaria rhabdocaeca nov. spec. (Fig. 9.)

Systematic position: furcocercous larva.

Parthenita: sporocyst.

Host: *Planorbis trivolvis* Say.

Habitat and time of collection: slough, Urbana, Ill., 1918.

Flame-cell formula: $\alpha + \beta + \gamma + \vartheta + \epsilon = 2 + 2 + 2 + 2 + 2$ or 10.

Forked-tailed cercaria with body 0.14 mm. long by 0.06 mm. wide, with tail trunk practically twice as long, and with furci 0.2 mm. in length. Anterior part of body provided with thick blunt spines. Acetabulum wanting; oral sucker pyriform, with larger end directed inward. Eye-spots wanting.

Sporocyst sacculate, with muscular pocket (not a digestive tract) and birthpore at anterior end.

Prepharynx present; pharynx only slightly muscular; esophagus short and inconspicuous; cecum single median. Mucin glands three to each side of the body, opening through individual ducts at sides of oral aperture. Duct pores capped with hollow piercing spines.

Excretory bladder simple, with collecting tubule emerging from each side. Flame cells consisting of five paired groups on each side of body.

A few large germ-gland cells located ventral to bladder.

SUMMARY.

1. The number and distribution of groups of flame cells is the fundamental basis of structure of the excretory system of the distomes. Number of flame cells in each group and total number of flame cells are of secondary significance.

2. The group of flame cells is typical of all members of a family or at least of a subfamily.

3. The basic flame-cell group of each family or subfamily may be expressed as a general formula. Substitution in this general formula for the exact number of flame cells in each group shows variations within individual units, which, in each case, are multiples of the common denominator on which the general formula is based.

4. Seven new species of distome cercariæ are described, which are of special importance in affording evidence of the orderliness of the excretory system of the Digenea.

REFERENCES CITED.

Cort, W. W.

'18 Homologies of the Excretory System of Forked-tailed Cercariæ. Jour. Parasit., 4, 49–57, 2 figs.

Faust, E. C.

'17 Notes on the Cercariæ of the Bitter Root Valley, Montana. Jour. Parasit., 3, 105–123, 1 pl.

'18 Life History Studies on Montana Trematodes. Ill. Biol. Monogr., 4, 1–120, 9 pl.

'18a Studies on Illinois Cercariæ. Jour. Parasit., 4, 93–110, 2 pl.

Looss, A.

'94 Die Distomen unserer Fische und Frösche. Bibl. Zoöl., 16, 1–296, 9 Taf.

'96 Recherches sur la faune parasitaire de l'Égypte. Mem. l'institute Égyptien, 3, 1–252, 16 pl.

THE EXCRETORY SYSTEM IN DIGENEA. III.

ERNEST CARROLL FAUST.

NOTES ON THE EXCRETORY SYSTEM IN A MONOSTOME LARVA, *Cercaria spatula* NOV. SPEC.[1]

In two previous papers of this series (Faust, 1919, 1919a) the writer has discussed the excretory system of an amphistome larva, *Cercaria convoluta*, and significant types of this system in distome larvæ. This paper presents data on the development and structure of the excretory system in a monostome larva, *Cercaria spatula* nov. spec. The system will be best presented by first tracing its development.

At the time when the cercaria germ-ball begins to differentiate and the tail portion becomes distinguishable from the body, the excretory system is recognizable as a pair of tubules running nearly the length of the animal. Soon the portion in the posterior part of the body proper draws together and fuses to form the bladder. Sometime after this the tail tubules coalesce to form the single median tubule of the mature larva. Meanwhile the tubules anterior to the bladder fuse with one another at their anteriormost ends, thus forming a circuitous tubule from one side to another.

At the earliest stage there is a single flame cell for each side of the body. This is situated at the anterior end of the body. Later this cell wanders backward along the lateral tubule so that at the time when the tubular circuit becomes closed the flame cell occupies a place halfway between the anterior end of the system and the bladder. It then divides into two flame cells, the fundaments of the system. The anterior one wanders forward and by a two-fold bifurcation gives rise to four flame cells. The posterior one wanders backward and by a single bifurcation forms two cells. Thus the flame-cell number of the mature cercaria amounts to 12, or 6 on each side of the median line (Fig. 2). Since the two cells on each side of the body, arising from the bifurcation of the original cell, are the key to the further development of the system, the general flame-cell formula may be represented as $\alpha' + \alpha'' = 4 + 2$ or 6.

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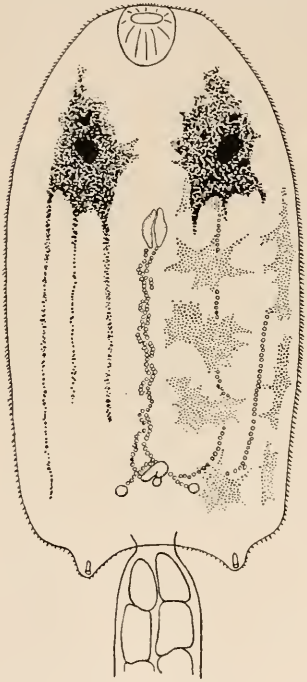
[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 132.

Data on the excretory system in the redia of *Cercaria spatula* furnish additional evidence in favor of the belief that this formula is valid for the excretory system in this species. In the very young redia a single flame cell is found on each side of the body, at the end of a coiled tubule which opens to the outside through an inconspicuous pore. Later a single bifurcation of the flame cell and the distal end of the coiled tubule occurs so that in the mature redia the two collecting tubules on each side open outward



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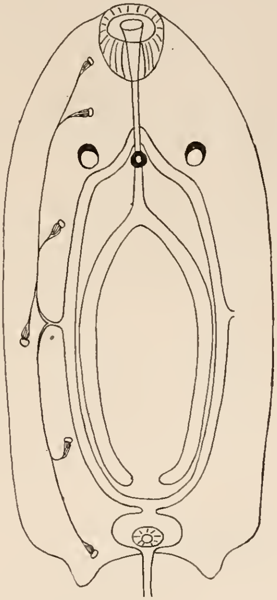

2

FIG. 1. *Cercaria spatula*, dorsal view, showing eye-spots, pigmentation, and genital cells. × 170.

FIG. 2. *C. spatula*, dorsal view, showing digestive and excretory systems. × 170.

through a single pore (Fig. 3). In the parthenita, then, as well as in the cercaria, the basis of the system is a two-fold one, as expressed in the formula $\alpha' + \alpha''$.

While many monostome cercaria have been described and in most cases the tubular circuit has been figured in the description, the lateral system or secondary tubule has been suggested only once, in *Cercaria robusta* (Faust, 1918). Omission of the more delicate part of the system is undoubtedly due to the ease with which the main mass of the system has been observed, since it is filled with granules, and the extreme difficulty with which the finer tubules are demonstrated. Since this same type of system is probably common to these two species, the formula $\alpha' + \alpha''$ is quite likely a common denominator for the family or subfamily to which they belong.

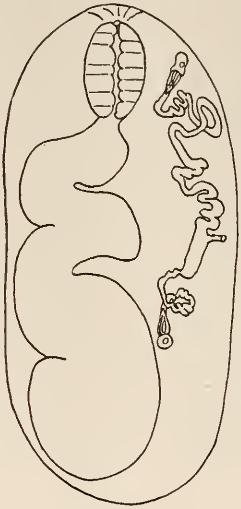

FIG. 3. Very young redia of *C. spatula*, showing digestive tract and flame-cell structure. × 75.

Cercaria spatula nov. spec. (Figs. 1–3.)

Systematic position: larval monostome, probably Notocotylidæ.

Parthenita: redia.

Host: *Physa gyrina* Say.

Habitat: Urbana, Illinois, 1918.
Flame-cell formula: $\alpha' + \alpha'' = 4 + 2$ or 6.

Monostome larva of spatulate outline, with a length of about 0.5 mm. and a width of nearly half that measurement; tail equally long and capable of great distension along its transverse axis and great extension along its longitudinal axis. Body entirely covered with minute spines. Trioculate, with dense masses of melanoidin pigment around the lateral eyes, extending caudad along the six posterior nerve trunks; integument of entire body more or less infiltrated with pigment granules. Locomotor pockets at posterior part of body, small, simple. Tail aspinose with six continuous pairs of large vacuolated cells at sides of caudal excretory canal.

Oral sucker leading into a long narrow esophagus; esophagus branching some little distance behind the median eye to form a typical furculum.

Excretory bladder ovate, transversely compressed, with a strong sphincter; main circuitous collecting tubule with a main branch on each side; flame cells anterior to main branch four posterior to main branch two.

Genital organs consisting of median ovary, paired testes in plane slightly behind ovary, uterus and vas deferens leading forward respectively to vagina and cirrus sac; vitellaria of two series of five inner and three outer glands on dorsal side of body.

Redia with long rhabdocœl gut, strong pharynx lined with transverse chitinous ribbing, and spines anterior and posterior to pharynx. Birthpore inconspicuous. Flame cells two for each side of the body.

Cystogenous granules many, densely opaque. Decaudation slow; encystment very slow.

SUMMARY.

1. Development of the excretory system in the monostome larva *Cercaria spatula* nov. spec. gives support to the principle of orderly development and conservatism of the system in monotome larvæ.

2. Comparison of the system in the cercaria and redia of this species yields evidence that there is a fundamental homology of the units in the hermaphroditic and the parthenogenetic generations of this fluke.

REFERENCES CITED.

Faust, E. C.

'18 Studies on Illinois Cercariæ. Jour. Parasit., 4, 93–110, 2 pl.

'19 The Excretory System in Digenea. I. Notes on the Excretory System of an Amphistome, *Cercaria convoluta* nov. spec. Biol. Bull., 36, 315–321.

'19a The Excretory System in Digenea. II. Observations on the Excretory System in Distome Cercariæ. Biol. Bull., 36, 322–339.

THE MARINE BIOLOGICAL LABORATORY

Twenty–First Report; for the Year 1918

Thirty–First Year

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I. TRUSTEES

EX OFFICIO

Frank R. Lillie, *Director*, The University of Chicago.
Gilman A. Drew, *Assistant Director*, Marine Biological Laboratory.
D. Blakely Hoar, *Treasurer*, 161 Devonshire Street, Boston, Mass.
Gary N. Calkins, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1922

Cornelia M. Clapp, Mount Holyoke College.
E. G. Conklin, Princeton University.
Ross G. Harrison, Yale University.
Camillus G. Kidder, 27 William Street, New York City.
M. M. Metcalf, Oberlin, Ohio.
William Patten, Dartmouth College.
Jacob Reighard, University of Michigan.
W. B. Scott, Princeton University.

TO SERVE UNTIL 1921

S. F. Clarke, Williamstown, Mass.
Charles A. Coolidge, Ames Building, Boston, Mass.

C. R. CRANE, Woods Hole, Mass., *President of the Corporation.*
ALFRED G. MAYOR, Carnegie Institution.
C. E. MCCLUNG, University of Pennsylvania.
T. H. MORGAN, Columbia University.
ERWIN F. SMITH, United States Department of Agriculture.
E. B. WILSON, Columbia University.

TO SERVE UNTIL 1920

H. H. DONALDSON, Wistar Institute of Anatomy and Biology.
M. J. GREENMAN, Wistar Institute of Anatomy and Biology.
C. W. HARGITT, Syracuse University.
H. S. JENNINGS, Johns Hopkins University.
GEORGE LEFEVRE, University of Missouri, *Secretary of the Board.*
A. P. MATHEWS, The University of Chicago.
G. H. PARKER, Harvard University.
HENRY B. WARD, University of Illinois.

TO SERVE UNTIL 1919

H. C. BUMPUS, Tufts College.
R. A. HARPER, Columbia University.
W. A. LOCY, Northwestern University.
JACQUES LOEB, The Rockefeller Institute for Medical Research.
GEORGE T. MOORE, Missouri Botanical Garden, St. Louis.
L. L. NUNN, Telluride, Colo.
W. J. V. OSTERHOUT, Harvard University.
JOHN C. PHILLIPS, 299 Berkeley Street, Boston, Mass.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the

President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT.

HENRY B. PIERCE,
[SEAL.] *Secretary of the Commonwealth.*

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alternation of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE TREASURER'S REPORT

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1918.

Assets.

Cash in bank	$ 7,503.52	
Petty cash at Woods Hole	200.00	$ 7,703.52
Notes receivable		1,000.00
Accounts receivable		10,938.88
Inventories (not available)		——
Items in suspense		11.73
Investments (Schedule I.)	$ 11,768.83	
Investment cash (Schedule II.)	485.20	12,254.03
Gansett property account	$ 22,317.20	
Less mortgage	14,629.21	7,687.99
Educational plant (Schedule III.):		
Land	$95,856.14	
Buildings	169,670.49	
Equipment	75,920.25	
	$341,446.88	
Less reserve for depreciation	21,140.24	320,306.64
		$359,902.79

Liabilities.

Bills payable		$ 90.58
Notes payable	$ 2,300.00	
Trust funds	9,954.03	12,254.03
		$ 12,344.61
Balancing account:		
Balance, January 1	$254,157.31	
Special donations	1,261.59	
Sundry adjustments	6.79	
	$355,425.69	
Less: Adjustment of depreciation reserve, 1917	$ 5,209.01	
Excess of expenses for year	2,658.50	
	$ 7,867.51	$347,558.18
		$359,902.79

MARINE BIOLOGICAL LABORATORY INCOME AND EXPENSES FOR YEAR ENDED DECEMBER 31, 1918

	Expense	Income	Loss	Gain
Administrative expense	$ 7,592.98		$ 7,592.98	
Bar Neck property expense	80.00		80.00	

BIOLOGICAL BULLETIN and annual dues	$ 2,341.57	$ 2,387.94		$ 46.37
Boat department	6,378.42		6,378.42	
Carpenter department	868.35	21.52	846.83	
Chemical department	1,436.27		1,436.27	
Club House expense	196.91		196.91	
Dexter House special expense	437.61		437.61	
Dormitory department	1,589.98	1,528.76	61.22	
Fish trap	1,323.62	1,360.10		36.48
Instruction	3,133.22	3,475.00		341.78
Interest on notes payable	150.00		150.00	
Lectures, evening	122.14		122.14	
Library department	1,492.70		1,492.70	
Maintenance, buildings and grounds	3,056.50		3,056.50	
Mess	19,672.60	21,016.28		1,343.68
New laboratory expense	3,245.28		3,245.28	
Newman cottage	49.19	175.00		125.81
Pumping station expense	378.07		378.07	
Research department		,3050.00		3,050.00
Sundry expense and income	1,807.57	2,519.41		711.84
Supply department	13,304.60	18,748.04		5,443.44
Truck	1,029.56		1,029.56	
Total current expenses	$69,687.14	$54,282.05	$26,504.49	$11,099.40
Total income	54,282.05		11,099.40	
Excess of expenses	$15,405.09		$15,405.09	
Depreciation	7,189.41			
Bad accounts charged off	64.00			
	$22,658.50			
Contribution by Friendship Fund, Incorporated	20,000.00			
Balance to balancing account	$ 2,658.50			

MARINE BIOLOGICAL LABORATORY INVESTMENTS (BOOK VALUES), DECEMBER 31, 1918.

Reserve Fund

$3,000 American Telephone & Telegraph Company, 4's (represented by receipts for same as collateral)	$2,921.25	
$500 Western Telephone & Telegraph Company 5's	496.88	
6 shares American Smelting & Refining Company, Preferred (receipt on file)	732.00	
8 shares General Electric Company	948.05	
14 shares United Shoe Machinery Company, Preferred	393.75	
5 shares Massachusetts Gas Companies, Preferred	444.63	$5,936.56

Lucretia Crocker Fund

$300 Liberty Loan Bonds, 4¼'s	$ 300.00	
⅕ of $1,000.00 American Telephone & Telegraph Company's 4's	194.75	

18 shares Vermont and Massachusetts Railroad Company	$2,416.50	
1 share American Telephone & Telegraph Company	125.02	
3 shares General Electric Company	364.85	
1 share West End Street Railway Company	83.00	3,484.12

Library Fund

$300 Liberty Loan Bonds, 4¼'s	$ 300.00	
$\frac{4}{5}$ of $1,000 American Telephone & Telegraph Company, 4's	779.00	
3 shares American Telephone & Telegraph Company	375.04	
1 share American Smelting & Refining Company Preferred (receipt on file)	122.00	
3 shares General Electric Company	362.10	
5 shares United Shoe Machinery Company, Preferred	140.63	
3 shares Massachusetts Gas Companies, Preferred	269.38	$2,348.15
Total, Exhibit A		$11,768.83

Harvey S. Chase & Company Certified Public Accountants,
84 State St., Boston

February 11, 1919

Mr. D. Blakeley Hoar,
161 Devonshire Street,
Boston.

Dear Sir: We have completed our audit of the accounts of the Marine Biological Laboratory for the year ended December 31, 1918, as kept both at your office in Boston and at Woods Hole, and report thereon in the accompanying exhibits and schedules:

Exhibit A—Balance-Sheet as of December 31, 1918.
B—Income-and-Expense for the year ended December 31, 1918.
Schedule I—Investments (Book Values).
II—Cash Receipts and Payments on account of Investments.
III—Summary of Inventory of Land, Buildings and Equipment.
IV—Depreciation Reserve.

We certify that, subject to the comments herewith, the balance-sheet and income-and-expense statement shown in Exhibits A and B are in accordance with the books and correct to the best of our knowledge and belief.

Very respectfully,
Harvey S. Chase & Company,
Certified Public Accountants.

V. STATEMENT CONCERNING THE LIBRARY

On account of the exceptional conditions of the state of war, it was found impossible to carry out any external enterprise, and in the absence of the assistant librarian, other work was limited. For these reasons the main effort was devoted to extension of the catalogues. In addition, a number of gifts were accessioned. Among these must be mentioned the gift by Miss Katharine Foot of about 750 reprints from her collection, expecially rich in cytology, and the gift by Dr. Ida H. Hyde of a most valuable collection of about 1,900 books and reprints on physiological subjects. These gifts are particularly valuable as they constitute research collections including many rare publications at least two thirds of which are new to the Library. The coöperation of investigators has been a most important factor in building up the library.

VI. THE DIRECTOR'S REPORT

January 1, 1919

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to present herewith a report of the thirty-first session of the Marine Biological Laboratory for the year 1918. Our anticipation both last season and this, that the war would affect our regular work unfavorably has been to a certain extent realized. But the conclusion of the war nevertheless finds us in a strong position, with our organization intact, and no actual suspension of our work incurred in spite of a considerable diminution of it.

If we examine the tabular view of attendance given under 3 beyond, we find that the total attendance for the last four years was: 1915, 242; 1916, 231; 1917, 212; 1918, 161. The attendance of investigators during the same period was cut down from 137 to 92, and of students from 105 to 69. The greatest proportional loss was among the younger investigators, whose numbers were reduced to less than one half. This is explained by the fact that the young men were serving our country in various technical capacities. There was a corresponding reduction in receipts from students and investigators: 1915, $8,325; 1916, $8,725; 1917, $7,850; 1918, $6,530. The receipts of the Supply Department were $18,748.04 as against $18,815.22 in 1917; $21,096.65 in 1916; and $16,932.00 in 1915.

It was possible to effect various economies corresponding to the somewhat smaller scale of operations, and the treasurer's report shows a net excess of receipts over expenditures for the year amounting to $4,594.91. Out of total expenditures of $69,687.14, Mr. Crane furnished $20,000.00 through the Friendship Fund, Inc., for which our best thanks seem singularly inadequate. The name of the Fund expresses the spirit of the giver, and the spirit of the Laboratory toward Mr. and Mrs. Crane in ever increasing degree if that is possible.

As stated in the last annual report,[1] shortly after beginning of the war the Laboratory leased to the United States of America for the Navy Department without compensation, the Mess-hall, laundry, kitchen and storehouse, the Homestead and the lecture hall building from September 15, 1917, to May 15, 1918. On the conclusion of this period the lease was renewed, with certain adjustments to cover the summer session, until June 30, 1919. The Navy Department so coöperated with us that we were able to continue our work without interruption. During the summer of 1918, the members of the Naval Reserve at Woods Hole were furnished board at the Mess which was run by the Laboratory as usual, and at the close of the summer session the Navy Department took it over again. The plan worked harmoniously, and I feel we owe an acknowledgment to the Navy personnel for the courtesy with which all elements of the situation were invariably met. Of the 200 members of the Naval Reserve at Woods Hole, one-half was furnished board at the Mess while the other half was on duty with the boats, and these sections alternated. Owing to conclusion of the armistice, the lease will be terminated from and after January 31, 1919.

The Laboratory is glad to have made the above small contribution to the winning of the war. Many of the members of the staff, the Board of Trustees, and of the Corporation have been concerned in war work, some by enlistment in various branches of the armed forces of the United States, others by service on various scientific boards, others again by undertaking specific pieces of investigation for the Government. As a consequence there were many absences from the staff and investigators in the summer. Those who were able to do so took up the work of the absent members, and recognition is due for the efficiency with which they conducted the work of the Laboratory.

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[1] Biological Bulletin, Vol. 35, p. 142.

The Tablet in memory of the First Director of the Laboratory was designed by Miss Frances Grimes of New York City, and was completed and set up in the vestibule of the brick building in the spring of 1918. It is of bronze, and bears the following inscription: "To the Memory of Charles Otis Whitman, 1842–1910, Professor of Zoölogy in Universities of Japan and the United States, 1877–1910. First Director of the Marine Biological Laboratory, 1888–1908. This tablet is erected by the Corporation and Board of Trustees of the Marine Biological Laboratory in grateful recognition of his inspiring Leadership, Devotion to the Spirit of Coöperation among Scientific Men, high ideals of Research, and of his great work in the Advancement of Biological Science."

May this memorial serve to keep green the memory of the man who more than any other, established the spirit of the Marine Biological Laboratory:

The death of Franklin P. Mall, Professor of Anatomy in Johns Hopkins Medical School occasioned a serious loss to the Laboratory. The following resolution has been entered in the minutes of the Board of Trustees:

"Franklin Paine Mall died at Baltimore, November 17, 1917, in his fifty-sixth year and just as he was entering on the most comprehensive phase of his work in human embryology.

"Since 1897 he had been a member of the Board of Trustees of the Marine Biological Laboratory.

"The aims and ideals of the Laboratory were close to his heart, and many young investigators who have studied at Woods Hole owe to his suggestion their impulse to do this.

"A student of broad biological training and still broader interests; a teacher of genius; a skilful organizer of scientific work and workers; an investigator with a peculiar gift for the appreciation of form and with a strong artistic sense; a whimsical counsellor, blending wise sayings with his common talk. . . . This Board owes much to him, and on this occasion desires to record its sorrow for his too early death and its appreciation of his qualities."

The vacancy in the Board of Trustees caused by the death of Professor Mall has been filled by the election of W. J. V. Osterhaut, Professor of Botany in Harvard University.

We record with sorrow the death from pheumonia on January 29, 1919, of Professor William E. Kellicott, head of the Department of Biology of the College of the City of New York and of the Department of Embryology of the Marine Biological Laboratory. During his long connection with our Laboratory, as investigator in 1903, instructor in embryology 1908–1914, and head of this department since 1915, Professor Kellicott won many friends and rendered most valuable service. His loss will be deeply felt by his friends and by the Laboratory.

War conditions prevented any considerable development of the Gansett property in 1918. The plans stated in the last report were however carried through. The Town of Falmouth accepted the main road (Whitman Road) and part of Gansett Road, which are now town roads, and purchased the water system installed by the Laboratory on these roads at cost. Two residences have been erected. The Laboratory has paid off $1,781.60 on the mortgage, and holds to the credit of the Gansett account $2,522.66 in cash, and a mortgage of $1000.00 on two of the lots with residence.

The Laboratory has come through a period of serious uncertainty without harm. Now that this period of suspense is over, we are confronted with an opportunity for usefulness greater by all that has been lost by Europe in the matter of scientific usefulness, and by the spirit of heightened scientific activity that has been aroused in America during the stirring times of war. It is perhaps not so much an opportunity as a duty to see that American institutions of learning compensate the world to some extent for what has been lost. In our field of work this duty and this opportunity are ours.

1. THE STAFF

1918

THE STAFF

Frank R. Lillie, *Director*, Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

Gilman A. Drew, *Assistant Director*, Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

Gary N. Calkins, Professor of Protozoölogy, Columbia University.

E. G. Conklin, Professor of Zoölogy, Princeton University.

Gilman A. Drew, Assistant Director, Marine Biological Laboratory.

George Lefevre, Professor of Zoölogy, The University of Missouri.

Frank R. Lillie, Professor of Embryology, The University of Chicago.

C. E. McClung, Professor of Zoölogy, University of Pennsylvania.

T. H. Morgan, Professor of Experimental Zoölogy, Columbia University.

E. B. Wilson, Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Caswell Grave, Associate Professor of Zoölogy, Johns Hopkins University. (Absent in 1918.)

W. C. Allee, Professor of Biology, Lake Forest College. In charge in 1918.

George A. Baitsell, Instructor in Biology, Yale University. (Absent in 1918.)

Robert Chambers, Jr., Cornell University Medical College.

G. S. Dodds, Assistant Professor of Zoölogy, University of Missouri.

W. J. Kostir, Instructor in Zoölogy and Entomology, Ohio State University.

Ann H. Morgan, Professor of Zoölogy, Mount Holyoke College.

Charles L. Parmenter, Research Fellow, University of Pennsylvania.

EMBRYOLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

William E. Kellicott, Professor of Biology, Goucher College (absent in 1918.)

ROBERT A. BUDINGTON, Professor of Zoölogy, Oberlin College, (In charge in 1918.)

CHARLES G. ROGERS, Professor of Comparative Physiology, Oberlin College.

HUBERT B. GOODRICH, Associate Professor of Zoölogy, Wesleyan University.

PHYSIOLOGY

I. INVESTIGATION

ALBERT P. MATHEWS, Professor of Physiological Chemistry, The University of Chicago. (Absent in 1918.)

RALPH S. LILLIE, Professor of Biology, Clark University.

HAROLD C. BRADLEY, Assistant Professor of Physiological Chemistry. University of Wisconsin.

II. INSTRUCTION

RALPH S. LILLIE, Professor of Biology, Clark University.

WALTER E. GARREY, Professor of Physiology, Tulane University. (Absent in 1918.)

FRANK P. KNOWLTON, Professor of Physiology, Syracuse University.

A. R. MOORE, Professor of Physiology, Rutgers College.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

EDWARD G. SPAULDING, Professor of Philosophy, Princeton University. (Absent in 1918.)

BOTANY

GEORGE T. MOORE, Director, Missouri Botanical Garden and Professor of Botany, Washington University.

IVEY F. LEWIS, Professor of Biology, University of Virginia.

WILLIAM D. HOYT, Associate Professor of Biology, Washington and Lee University.

LIBRARY

H. MCE. KNOWER, Professor of Anatomy, University of Cincinnati. Librarian.

MARY E. SCOTT, Assistant Librarian.

CHEMICAL SUPPLIES[1]

OLIVER S. STRONG, Assistant Professor of Neurology, College of Physicians and Surgeons, New York City, Chemist.

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[1] Compound microscopes, with two oculars and two objectives, rack and pinion and fine adjustment, may be rented for the season at $7.00 each provided notice is received by the assistant director not later than June 15.

SUPPLY DEPARTMENT

G. M. Gray, Curator.
John J. Veeder, Captain.
E. M. Lewis, Engineer.
A. W. Leathers, Collector.
A. M. Hilton, Collector.
J. McInnis, Collector.
F. G. Gustafson, Collector in Botany.
Edna E. Wells, Clerk.

F. M. MacNaught, Business Manager.
Herbert A. Hilton, Superintendent of Buildings and Grounds.

2. INVESTIGATORS AND STUDENTS

1918

ZOÖLOGY

Independent Investigators

Addison, William H. F., Assistant Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, Warder C., Professor of Biology, Lake Forest College.
Allen, Ezra, Research Fellow, University of Pennsylvania.
Altenburg, Edgar, Instructor in Biology, Rice Institute.
Ando, Hidezo, University of Kyoto, Japan.
Beckwith, Cora J., Associate Professor of Zoölogy, Vassar College.
Bigney, Andrew J., Assistant in Zoölogy, Harvard University.
Boring, Alice M., Associate Professor of Zoölogy, University of Maine.
Bridges, Calvin B., Research Assistant, Columbia University.
Budington, Robert A., Professor of Zoölogy, Oberlin College.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Carothers, E. Eleanor, Assistant in Zoölogy, University of Pennsylvania.
Clapp, Cornelia M., Emeritus Professor of Zoölogy, Mount Holyoke College.
Clark, Eliot R., Professor of Anatomy, University of Missouri.
Clark, Eleanor L., Columbia, Mo.
Copeland, Manton, Professor of Biology, Bowdoin College.
Cowdry, Edmund V., Professor of Anatomy, Peking Union Medical College.
Crampton, H. E., Professor of Zoölogy, Barnard College.
Dexter, John S., Assistant Professor of Zoölogy, University of Saskatchewan.
Dodds, Gideon S., Assistant Professor of Zoölogy, University of Missouri.
Donaldson, Henry H., Professor of Neurology, Wistar Institute.
Drew, Gilman A., Assistant Director, Marine Biological Laboratory, Woods Hole.
Glaser, Otto C., Professor of Biology, Amherst, Mass.
Goodrich, Hubert B., Associate Professor of Zoölogy, Wesleyan University.
Gould, Harley N., Assistant Professor, West Virginia University Medical School.
Gregory, Louise H., Assistant Professor of Zoölogy, Barnard College.
Kindred, James E., Graduate Fellow, University of Illinois.
Knower, Henry McE., Professor of Anatomy, University of Cincinnati.
Lillie, Frank R., Chairman Department of Zoölogy, University of Chicago.

McClung, Clarence E., Director of Zoölogical Laboratory, University of Pennsylvania.

Mohr, Otto L., Prosector, University of Kristiania.

Miner, Roy W., Associate Curator of Invertebrate Zoölogy, American Museum of Natural History.

Morgan, Ann H., Professor of Zoölogy, Mount Holyoke College.

Morgan, Thomas H., Professor of Experimental Zoölogy, Columbia University.

Morrill, Charles V., Instructor in Anatomy, Cornell Medical College.

Muller, Hermann J., Instructor, Rice Institute.

Nabours, Robert K., Professor of Zoölogy, Kansas Agricultural College.

Packard, Charles, Instructor in Zoölogy, Columbia University.

Parker, George H., Professor of Zoölogy, Harvard University.

Parmenter, Charles L., Research Fellow, University of Pennsylvania.

Patten, William, Professor of Biology, Dartmouth College.

Patten, Bradley M., Assistant Professor of Histology and Embryology, Western Reserve University.

Plough, Harold H., Instructor in Biology, Amherst College.

Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.

Stockard, Charles R., Professor of Anatomy, Cornell University Medical College.

Strong, Oliver S., Assistant Professor of Neurology, Columbia University.

Sturtevant, Alfred H., Research Assistant, Columbia University.

Warren, Howard C., Stuart Professor of Psychology, Princeton University.

Whiting, Phineas W., Harrison Research in Zoölogy, University of Pennsylvania.

Wilson, Edmund B., Professor of Zoölogy, Columbia University.

Woodward, Alvalyn E., Instructor, Simmons College.

Beginning Investigators

Adams, A. Elizabeth, Instructor in Zoölogy, Mount Holyoke College.

Carlson, C. O., Professor of Biology, Doane College.

Collett, Mary E., Fellow in Zoölogy, University of Pennsylvania.

Davies, I. J., Technician, Rice Institute.

Donovan, Stephen M., Professor of Biology, St. Bonaventure's College.

Goodrich, Clara C., Middletown, Conn.

Hedge, Mabel L., Technician, Columbia University.

Howland, Ruth B., Associate Professor of Biology, Sweet Briar College.

Kostir, W. J., Instructor in Zoölogy, Ohio State University.

Matsui, Kenkichi, Agricultural College of Morioka, Japan.

Nonidez, Jose, F., Professor of Zoölogy, University of Murcia, Spain.

Olsen, Chris E., Preparator, American Museum of Natural History.

Schrader, Franz, Assistant, Columbia University.

Shimotori, Show, Artist, American Museum of Natural History.

Starr, Isaac, Jr., University of Pennsylvania.

Wallace, Edith M., Research Assistant, Columbia University.

PHYSIOLOGY

Independent Investigators

Chambers, Robert Jr., Instructor in Anatomy, Cornell University Medical College.

Clowes, George H. A., Biological Chemist, New York State Institute for Study of Malignant Diseases.

Dernby, Karl G., Rockefeller Institute.
Edwards, Dayton J., Lecturer in Physiology, Cornell University Medical College.
Harvey, E. Newton, Assistant Professor of Physiology, Princeton University.
Harvey, Mrs. E. Newton, Princeton, N. J.
Hecht, Selig, Assistant Professor of Physiology, Creighton University Medical School.
Hyde, Ida h., Professor of Physiology, University of Kansas.
Just, Ernest E., Professor of Physiology, Howard University.
Knowlton, Frank P., Professor of Physiology, Syracuse University.
Lillie, Ralph S., Professor of Biology, Clark University.
Loeb, Jacques, Head of Department of Experimental Biology, Rockefeller Institute for Medical Research.
Moore, Arthur R., Professor of Physiology, Rutgers College.
Moore, Mrs. Arthur R., New Brunswick, N. J.
Redfield, Alfred C., Instructor in Physiology, Harvard Medical School.
Spaeth, Reynold, A., Associate in Physiological Hygiene, Baltimore, Md.

Beginning Investigators

Banus, Mario G., Rockefeller Institute.
Bright, Elizabeth M., Technician, Harvard Medical School.
Irwin, Marian, Graduate Student, Radcliffe College.

BOTANY

Independent Investigators

East, Edward M.. Professor Experimental Plant Morphology, Harvard University.
Hoyt, William D., Associate Professor of Biology, Washington and Lee University.
Lewis, Ivey F., Professor of Biology, University of Virginia.
Moore, George T., Director, Missouri Botanical Garden.
Osterhout, Winthrop J. V., Professor of Botany, Harvard University.

Beginning Investigators

Brooks, Mrs. S. C., 333 Longwood Ave., Boston, Mass.
Thomas, Helen S., Radcliffe College.

STUDENTS

1918

ZOÖLOGY

Abrams, Edith H., Assistant in General Zoölogy, Mount Holyoke College.
Baldwin, Eva E., Goucher College.
Brown, Mary, Student, Butler College.
Clark, Rosamond, 40 Commonwealth Ave., Boston, Mass.
Clarke, Alice E., Student, Goucher College.
Closson, Mary B., Student, Ursinus College.
Colcord, Edward C., Student, Oberlin College.
Daboll, Mary, Student, Mount Holyoke College.

Ebeling, Karl W., Student, Johns Hopkins University.
Elliott, Grace E., Knox College.
Flaig, Julian V., Student, Princeton University.
Gates, Gordon E., Student, Colby College.
Greene, Charlotte L., Student, Barnard College.
Hanson, Frank B., Instructor, Washington University.
Hyde, Alice, Student, Mount Holyoke College.
Jones, Harold E., Assistant, Amherst College.
Koerner, Sophie, Student, Barnard College.
Krafka, Elizabeth B., Laboratory Assistant, Lake Forest College.
Kranz, Aletha, Student, University of Chicago.
Lagemann, Anna E., Student, Barnard College.
Lillie, Catherine C., Student, Vassar College.
Lupo, Patsy, Mount Holyoke College.
McCash, Gladys K., Student, Mount Holyoke College.
McClay, Edith L., Student, Mount Holyoke College.
Mason, Eleanor D., Student, Mount Holyoke College.
Meade, Richard H., Jr., University of Virginia.
Metcalf, Rachel V., Oberlin College.
Mulheron, Helen, Teacher of English, St. Michaels School.
Mullins, Mary R., Hunter College.
Oak, Dorothy, Student, Barnard College.
Polk, Rebeka, Student, Mount Holyoke College.
Powis, Ethel M., Student, Mount Holyoke College.
Price, Genevieve, Student, Oberlin College.
Seaver, Charlotte, Sweet Briar College.
Shonka, Emil F., Student, St. Procopius College.
Stewart, Walter B., Student, Princeton University.
Thompson, Martha L., Student, Knox College.
Trotter, Mildred, Student, Mount Holyoke College.
Wheatley, Marjorie A., Student, Vassar College.
White, Frances W., Student, Oberlin College.
Willmann, Edith, Student, Barnard College.

EMBRYOLOGY

Abt, Arthur F., Student, University of Chicago.
Agersborg, H. P. K., Tutor, College of City of New York.
Brown, Alice L., Assistant Instructor, University of Kansas.
Cauldwell, Katharine, Student, Bryn Mawr College.
Hosford, Caroline A., Instructor in Zoölogy, Doane College.
Hughes, Sally P., Instructor, Grinnell College.
Hughes, J. Arthur, Student, Carleton College.
Miller, Erwin C., Student, Dartmouth College.
Norris, Henry M., Student, Princeton University.
Seago, Mary M., Student, Sophie Newcomb College.
Stedman, Hazeltene L., Student, Mount Holyoke College.
Wolff, Dorothy, Assistant in Comparative Anatomy in Mount Holyoke College.

PHYSIOLOGY

Baskervill, Margaret L., Instructor in Biology, Mississippi Industrial Institute and College.

Brokaw, Eunice, Student, Knox College.

Fulton, B. B., Assistant Entomologist, New York Agricultural Experiment Station.

Green, Susan A., Professor of Biology, Maryville College.

Hinrichs, Marie A., Student, University of Chicago.

Holland, Dorothy F., Mount Holyoke College.

Martin, Bertha E., Assistant Professor of Biology, Wheaton College.

Montgomery, Priscilla B., University of Pennsylvania.

Spencer, Helen W., Student, Radcliffe College.

Streeseman, Adele E., Instructor in Physics, Vassar College.

BOTANY

Beyer, Henry G., U. S. Navy, Retired.

Chadbourne, Joseph H., Jr., 11 Irvington St., Waban, Mass.

Fessenden, Anna P., Smith College.

Fouts, Janet W., Barnard College.

Jewett, Gladys E., Student, Wheaton College.

Tuttle, Gurynethe, M., Instructor in Botany, University of Alberta.

3. TABULAR VIEW OF ATTENDANCE

	1914	1915	1916	1917	1918
Investigators—Total	129	137	129	129	93
Independent:					
Zoölogy	62	69	70	63	51
Physiology	22	20	23	23	16
Botany	10	6	7	8	5
Under Instruction:					
Zoölogy	31	36	25	24	16
Physiology	1	4	3	6	3
Botany	3	2	1	5	2
Students—Total	89	105	102	83	69
Zoölogy	43	47	50	46	41
Embryology	21	37	26	16	12
Physiology	10	15	14	13	10
Botany	15	6	12	8	6
Total Attendance	218	242	231	212	162
Institutions Represented—Total	77	79	73	77	72
By investigators	51	59	51	60	49
By students	47	42	45	36	38
Schools and Academies Represented.					
By investigators	1	3	–	2	–
By students	5	9	3	5	–

4. SUBSCRIBING INSTITUTIONS—1918

Amherst College
Barnard College
Bryn Mawr College
Carleton College
Columbia University
Cornell University Medical College
Creighton University
Dartmouth College
Else Seringhaus Scholarship, Hunter College, New York City
Goucher College
Harvard University
Harvard University Medical School
Johns Hopkins University
Kansas State Agricultural College
Lake Forest College
Mount Holyoke College
Oberlin College
Princeton University
Radcliffe College
Rice Institute
Rockefeller Institute for Medical Research
Smith College
Sophie Newcomb College
University of Chicago
University of Illinois
University of Kansas
University of Michigan
University of Pennsylvania
University of Wisconsin
Vassar College
Wheaton College
Wistar Institute of Anatomy and Biology
Yale University

SCHOLARSHIP TABLES

Lucretia Crocker Scholarships for Teachers in Boston
Scholarship of $100, Supported by a friend of the Laboratory since 1898.

5. EVENING LECTURES, 1918

Friday, July 5,
Prof. E. M. East.........."The Phenomenon of Self-Sterility."
Tuesday, July 9,
Prof. W. J. V. Osterhout .."Photosynthesis."
Friday, July 12,
Prof. Jacques Loeb........"Why Does Mutilation of an Organism Induce Regeneration?"
Tuesday, July 16,
Dr. S. O. Mast............."Problems, Methods and Results in Behavior."
Friday, July 19,
Prof. T. H. Morgan........"Secondary Sexual Characters, Sex-linked Characters and Gynandromorphs."
Tuesday, July 23.
Dr. Jules Duesberg......."Mitochondria."
Friday, July 26,
Dr. R. S. Lillie..........."Nature of Protoplasmic Transmission."
Tuesday, July 30,
Dr. Roy W. Miner........."From Tidal Zone to Museum."
Friday, Aug. 2,
Dr. Gary N. Calkins........"*Uroleptus mobilis* and the Problem of Conjugation."
Tuesday, Aug. 6,
Prof. W. M. Wheeler......"The Life History and Behavior of the Worm-lion."
Friday, Aug. 9,
Dr. J. Arthur Harris......"Mathematics in Biology."
Tuesday, Aug. 13,
Prof. Ross G. Harrison...."Experiments on the Development of Limbs."

6. MEMBERS OF THE CORPORATION

Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.

Carey, Mr. Arthur Astor, Fayerweather St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Davis, Major Henry M., Syracuse, N. Y.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Farlow, Prof. W. G., Harvard University, Cambridge, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, 955 Park Ave., New York City, N. Y.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kennedy, Mr. Geo. G., 284 Warren St., Roxbury, Mass.
Kidder, Mr. C. G., 27 William St., New York City, N. Y.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
Marrs, Mrs. Laura Norcross, 9 Commonwealth Ave., Boston, Mass.
Mason, Miss E. F., 1 Walnut St., Boston, Mass.
Mason, Miss Ida M., 1 Walnut St., Boston, Mass.
Means, Mr. James Howard, 196 Beacon St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Columbia University, New York City, N. Y.
Morgan, Mrs. T. H., New York City, N. Y.
Noyes, Miss Eva J.

Nunn, Mr. Lucian L., Telluride, Colo.
Osborn, Prof. Henry F., American Museum of Natural History, New York City, N. Y.
Phillips, Dr. John C., Windy Knob, Wenham, Mass.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Pulsifer, Mr. W. H., Newton Center, Mass.
Rogers, Miss A. P., 5 Joy St., Boston, Mass.
Sears, Dr. Henry F., 86 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Smith, Mrs. C. C., 286 Marlboro St., Boston, Mass.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Whitney, Mr. Henry M., Brookline, Mass.
Wilcox, Miss Mary A., Wellesley College, Wellesley, Mass.
Wilmarth, Mrs. H. D., Elliott St., Jamaica Plain, Mass.
Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.
Wilson, Prof. W. P., Commercial Museum, Philadelphia, Pa.

7. CORPORATION MEMBERSHIP LIST

August, 1918

Abbott, Prof. J. F., Washington University, St. Louis, Mo.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.
Adams, Miss A. E., Mount Holyoke College, South Hadley, Mass.
Agersborg, Dr. H. P. K., College of the City of New York, New York City, N. Y.
Allee, Dr. W. C., Lake Forest College, Lake Forest, Ill.
Allen, Prof. Ezra, 125 Thompson Ave., Ardmore, Pa.
Allyn, Miss Harriet M., Hackett Medical College, Canton, China.

ALSBERG, DR. C. S., U. S. Dept. of Agriculture, Washington, D. C.
ALTENBURG, DR. EDGAR, Rice Institute, Houston, Texas.

BAITSELL, DR. GEORGE A., Yale University, New Haven, Conn.
BAKER, MRS. L. D., 123 Chiswick Road, Boston, Mass.
BAKER, DR. E. H., American Institute of Science, Chicago, Ill.
BANCROFT, PROF. F. W., Aloha Farm, Concord, Calif.
BECKWITH, MISS CORA J., Vassar College, Poughkeepsie, N. Y.
BEHRE, MISS ELINOR H., Carnegie Institution of Washington, Cold Spring Harbor, Long Island.
BEYER, DR. H. G., The Marlborough, Washington, D. C.
BIGELOW, PROF. M. A., Teachers College, Columbia University, New York City.
BIGELOW, PROF. R. P., Mass. Institute of Technology, Cambridge, Mass.
BINFORD, DR. RAYMOND, Earlham College, Richmond, Ind.
BORING, MISS ALICE M., Union Medical College, Peking, China.
BOX, MISS CORA MAY, University of Cincinnati, Cincinnati, Ohio.
BRADLEY, DR. HAROLD C., University of Wisconsin, Madison, Wis.
BRUMFIEL, DR. DANIEL M., University of Iowa, Iowa City, Iowa.
BUCKINGHAM, MISS EDITH N., 342 Marlboro St., Boston, Mass.
BUDINGTON, PROF. R. A., Oberlin College, Oberlin, Ohio.
BUMPUS, PROF. H. C., Tufts College, Tufts College, Mass.
BYRNES, DR. ESTHER F., 193 Jefferson Ave., Brooklyn, N. Y.

CALKINS, PROF. GARY N., Columbia University, New York City, N. Y.
CALVERT, PROF. PHILIP P., University of Pennsylvania, Philadelphia, Pa.
CARLSON, PROF. A. J., University of Chicago, Chicago, Ill.
CAROTHERS, MISS ELEANOR E., University of Pennsylvania, Philadelphia, Pa.
CARVER, PROF. GAIL L., Mercer University, Macon, Ga.
CARY, DR. L. R., Princeton University, Princeton, N. J.

Casteel, Dr. D. B., University of Texas, Austin, Texas.
Cattell, Prof. J. McKeen, Garrison-on-Hudson, N. Y.
Cattell, Mr. McKeen, Garrison-on-Hudson, N. Y.
Chambers, Dr. Robert, Jr., Cornell University Medical College, New York City, N. Y.
Chester, Prof. Webster, Colby College, Waterville, Maine.
Chidester, Prof. F. E., Rutgers College, New Brunswick, N. J.
Child, Prof. C. M., University of Chicago, Chicago, Ill.
Clapp, Prof. Cornelia M., Mount Holyoke College, South Hadley, Mass.
Clark, Prof. E. R., University of Missouri‘ Columbia, Mo.
Clowes, Dr. G. H. A., State Cancer Laboratory, Buffalo, N. Y.
Coe, Prof. W. R., Yale University, New Haven, Conn.
Cohn, Dr. Edwin J., 26 Walker St., Cambridge, Mass.
Cole, Dr. Leon J., College of Agriculture, Madison, Wis.
Colley, Dr. R. H., Dartmouth College, Hanover, N. H.
Colton, Prof. H. S., Ardmore, Pa.
Coolidge, Mr. C. A., Ames Building, Boston, Mass.
Copeland, Prof. Manton, Bowdoin College, Brunswick, Maine.
Coutant, Mrs. Mary W., 1058A Sterling Place, Brooklyn, N. Y.
Cowdry, Dr. E. V., Union Medical College, Peking, China.
Crampton, Prof. H. E., Barnard College, Columbia University, New York City, N. Y.
Crane, Mrs. C. R., Woods Hole, Mass.
Curtis, Prof. W. C., University of Missouri, Columbia, Mo.

Danchakoff, Mme. Vera, College of Physicians and Surgeons, New York City, N. Y.
Davis, Mr. Donald W., College of William and Mary, Williamsburg, Va.
Davis, Prof. Bradley M., University of Pennsylvania, Philadelphia, Pa.
Derick, Prof. Carrie M., McGill University, Montreal, Canada.
Dexter, Dr. J. S., University of Saskatchewan, Saskatoon, Saskatchewan.
Dodds, Prof. G. S., Medical School, University of West Virginia, Morgantown, W. Va.

DONALDSON, PROF. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

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BIOLOGICAL BULLETIN

THE DEVELOPMENT OF THE CASTES OF NINE GENERA AND THIRTEEN SPECIES OF TERMITES.

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In a former paper (Thompson, 1917), the writer showed that the newly hatched nymphs of the termite *Reticulitermes flavipes* Kol., 1.1 mm. long, although externally all alike, are differentiated by internal structural characters into two distinct types—(*a*) reproductive nymphs and (*b*) worker-soldier nymphs—from which develop (*a*) the three fertile adult castes, the reproductive forms, and (*b*) the two sterile adult castes, the workers and the soldiers. It was further shown that when the reproductive nymphs have attained a length of 1.3–1.4 mm. they are differentiated by internal characters into "nymphs of the first form" and "nymphs of the second form," which develop finally into two of the three adult reproductive castes;[1] at a much later period, when they have attained a length of about 3.75 mm., the worker-soldier nymphs become differentiated by internal characters into the worker and the soldier nymphs.

Bugnion (1912, '13) states that the soldier (nasutus) of *Eutermes lacustris*, a new termite species from Ceylon, is differentiated at the time of hatching, and may be distinguished from the other newly hatched nymphs by both external and internal structures, namely: the frontal process (corne frontale) and the large frontal gland.

These observations of Bugnion are not in accord with the work of Knower (1894) who states that the nasutus of *Eutermes rippertii* (?)—since identified by Mr. N. Banks as *E. pilifrons*

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[1] The development of the third, wingless, reproductive caste of *R. flavipes* has not been worked out.

Holmgren—arises late in its development by molting from an antecedent worker-like form with thirteen antennal segments.

In view of these conflicting observations it seemed at first possible that different degrees of differentiation of the newly hatched nymphs, as well as differences in development, might exist in the various genera of termites, and perhaps even among the species of a genus, as, for example, in the genus *Eutermes;* the nasutus of *E. lacustris* being clearly defined at the time of hatching, according to Bugnion; the nasutus of *E. pilifrons* arising rather late in the development by molting from a worker-like form, according to Knower. But, before coming to any conclusion, it seemed desirable to have further data in regard to the conditions among other termites at the time of hatching and in the early stages of development.

The present study of the newly hatched and the developing nymphs of nine genera and thirteen species of American termites, from both temperate and tropical regions, was therefore undertaken for two reasons: first, to ascertain the conditions among different genera of termites, and second, to determine whether the development may vary within a genus, by comparing the conditions in *Eutermes lacustris*, as described by Bugnion, with those of *Eutermes pilifrons*, the Jamaican species observed by Knower, and with two other tropical species, *E. morio* Latreille, and *E. sanchezi* Holmgren.

MATERIAL.

Dr. Knower has very kindly sent me an abundance of preserved material of *Eutermes pilifrons*, consisting of eggs, young nymphs, winged adult reproductive forms, and adult workers and nasuti. I am indebted to Mr. N. Banks, of the Museum of Comparative Zoölogy, Cambridge, Mass., for the identification of this species.

Most of my material was furnished me by the Bureau of Entomology of the U. S. Department of Agriculture, and much of it was collected and fixed for me by Mr. T. E. Snyder, to whom my sincere thanks are due. The eggs and young of *Termopsis* I have collected myself at Pacific Grove, Cal., during a visit to the Hopkins Marine Station.

Gilson's fluid has proved a good fixative for the newly hatched nymphs, and Bouin's fluid is excellent for all older forms. As in the case of *Reticulitermes* whole mounts of the newly hatched and developing nymphs, stained with Conklin's picro-hæmatoxylin, are most useful for preliminary study.

The termites to be described in this paper are *Termopsis angusticollis* Walker, *Calotermes* n. sp. Banks, *Cryptotermes cavifrons* Banks, *Neotermes castaneus* Burm., *Arrhinotermes simplex* Hagen, *Reticulitermes flavipes* Kollar, *R. virginicus* Banks, and *R.* n. sp. Banks, *Anoplotermes fumosus* Müller, *Amitermes tubiformans* Buckley, *Eutermes pilifrons* Holmgren, *E. morio* Latreille and *E. sanchezi* Holmgren.

Family.	Genus and Species.	Length, Egg, Mm.	Length, Newly-hatched Nymphs, Mm.	Length, Small and Large Headed Nymphs, Mm.	No. Antennal Segments, Newly Hatched Nymphs.
Protermitidæ	*Termopsis angusticollis*	1.3 –1.7	?	2.8–3	?
	Calotermes n. sp.	1.2 –1.4	1.6	1.8–2	9, third entire.
	Cryptotermes cavifrons	1.2 –1.3	1.4 –1.6	2.4	9, third entire.
	Neotermes castaneus	1.6	?	2.6	?, probably 9.
Mesotermitidæ.	*Arrhinotermes simplex*	0.8 –1	1.2	2	9, third subdivided.
	Reticulitermes flavipes	0.68–0.7	1.1	2	9, third subdivided.
Metatermitidæ.	*Anoplotermes fumosus*	0.56–0.6	?	1.8–2	?, probably 11.
	Amitermes tubiformans	0.56–0.64	0.95–1.0	1.6–1.8	11, third subdivided.
	Eutermes pilifrons	0.64–0.7	0.8 –0.9	1.8–2	11, third subdivided.

In the accompanying table it may be seen that the first four genera belong to the family Protermitidæ of Holmgren, the fourth, together with *Reticulitermes*, belongs to the Mesotermitidæ, and the last three are Metatermitidæ. The present paper therefore includes examples from each family of the order Isoptera.

My study includes for each genus (1) the eggs, (2) the newly hatched nymphs, in some cases these have been actually dissected out from the egg shell, in other cases they are slightly older; (3) an early stage of development, usually 0.2–0.4 mm. longer than the newly hatched nymphs, in which the external appearance, and especially the size of the heads, is similar in all nymphs; (4) a slightly older stage, varying considerably in length in the different genera, in which an external differentiation may be noted in the size of the heads, dividing the developing nymphs into "small headed" (reproductive) forms, and "large headed" (sterile) forms.

PROTERMITIDÆ.

Calotermes n. sp. Banks.—The eggs of the most primitive termites, those belonging to the family Protermitidæ, are the largest of the order Isoptera.

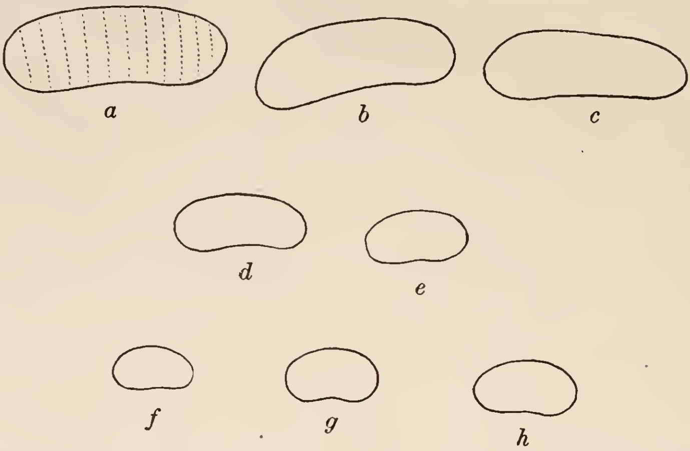

FIG. 1. Termite eggs. *a*, *Termopsis angusticollis; b*, *Calotermes* n. sp.; *c*, *Cryptotermes cavifrons; d*, *Arrhinotermes simplex; e*, *Reticulitermes flavipes; f*, *Anoplotermes fumosus; g*, *Amitermes tubiformans; h*, *Eutermes pilifrons.* Spencer Oc. 6, obj. 32, reduced one third.

The eggs of *Calotermes* n. sp. (Fig. 1, *b*) are long, slender, and reniform, and are slightly larger at one end, the future head end; they measure, in preserved specimens, from 1.2–1.4 mm. in length. In all the species of termites here described the egg length varies several tenths of a millimeter. It would be interesting to know whether these variations in length are constant in the living eggs, and whether they represent two different sizes of eggs, corresponding to the two different types of nymphs which are hatched. Since this paragraph was written further study of living eggs enables me to state with certainty that there are marked differences in size in the living eggs of a given species and, furthermore, among eggs in the same phases of development.

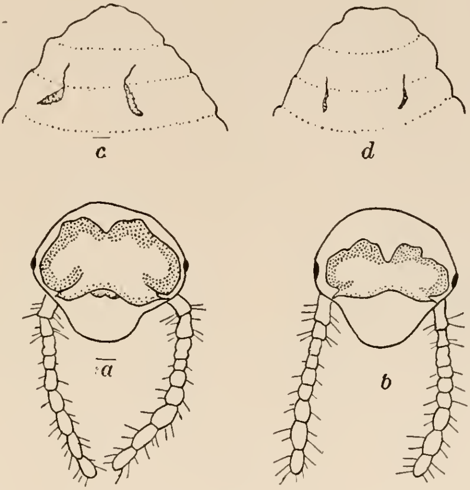

FIG. 2. *Calotermes* n. sp., newly hatched nymphs. *a*, head of reproductive nymph with large brain; *b*, head of soldier nymph with small brain; *c*, testes, reproductive nymph; *d*, testes, soldier nymph. Oc. 6, obj. 16, reduced one third.

The newly hatched nymphs of *Calotermes* n. sp. are 1.6 mm. long, with nine antennal segments, the third segment entire and bare. In size, shape and general external appearance these newly hatched nymphs are all alike, but, as in the genus *Reticulitermes*, they are separable by means of internal structures into two types: (*a*) nymphs with a large brain that almost fills the cavity of the head, large sex organs, and a white dense abdomen, the reproductive or fertile type (Fig. 2, *a*, *c*); and (*b*) nymphs with a smaller brain that does not nearly fill the head cavity, smaller sex organs, and a transparent abdomen, the soldier,[1] or sterile type (Fig. 2, *b*, *d*).

When the *Calotermes* nymphs have attained a body length of 1.8–2 mm. a marked difference in the size of the heads is noted,

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[1] The worker caste is lacking in the genus *Calotermes*.

the fertile, or reproductive type having a small head with a larger brain, the "small-headed" nymph of Grassi (1896–97); the sterile, or soldier type, with larger head and smaller brain, the "large-headed" nymph of Grassi. The number of antennal segments in these older nymphs is still nine, although the third segment is deeply grooved and subdivided into two parts. The increase in the number of antennal segments is therefore slightly slower than in the genus *Reticulitermes*, the "small-headed" and "large-headed" nymphs of *Reticulitermes*, 2 mm. in length, having twelve clearly defined antennal segments.

Termopsis angusticollis Walker.—*Termopsis angusticollis* produces the largest egg of any American termite. In form, the eggs are long, slender, and reniform, slightly larger at the future head end, and sometimes with surface markings (Fig. 1, *a*). Both in the living eggs and after fixation, and the eggs shrink very little in Gilson's Fluid, two fairly distinct sizes of eggs may be observed. The smallest are about 1.3 mm. long, the largest range from 1.5–1.7 mm. These size differences are independent of embryonic growth, since a large and a small egg may be in the same phase of development. I am unable to state what types of nymphs may hatch from the different sized eggs, for after searching repeatedly in many colonies in Pacific Grove, Cal., I do not feel sure that I have seen the newly hatched nymphs. The smallest nymphs that I have found are 2.2 and 2.5 mm. long, with eleven and twelve antennal segments. These may be the newly hatched forms, but their size in relation to the egg, their activity, for newly hatched nymphs are usually sluggish, as well as the eleven or twelve antennal segments, inclines me to believe that they have been hatched for a short time. In one embryo with well formed appendages nine segments could be distinguished in the antennae. I am therefore unable to make any definite statement regarding the body length and the number of antennal segments of the newly hatched nymphs of *Termopsis angusticollis*, although from the size of the egg I should expect the nymphs to measure about 1.6–1.9 mm. in length, and the number of antennal segments to be like that of the other Protermitidæ, *Calotermes* and *Cryptotermes*, namely, nine, with the third segment either entire or subdivided.

The youngest nymphs of *T. angusticollis* that I have examined —2.2–2.5 mm. long, with eleven and twelve antennal segments —are all alike in external macroscopic appearance, but with a lens the two types of fertile or reproductive nymphs and sterile or soldier[1] nymphs may be seen. The heads are of similar size, but the large brain almost filling the head cavity of the reproductive nymph (Fig. 3, *a*) is clearly distinguished from the smaller brain of the soldier nymphs (Fig. 3, *b*), and correlated with the brain structure is the whiter denser abdomen of the reproductive nymphs and the more transparent abdomen of the soldier nymphs. In stained and mounted specimens the larger sex organs of the reproductive nymphs are in marked contrast to the smaller ones of the soldier nymphs. The brain of all the adult castes of *T. angusticollis* has a very characteristic form, as if it had been pulled out laterally, due to the lateral extension of the optic lobes which extend out at right angles to the long axis of the body. This peculiar form of the brain is recognizable even in the youngest nymphs.

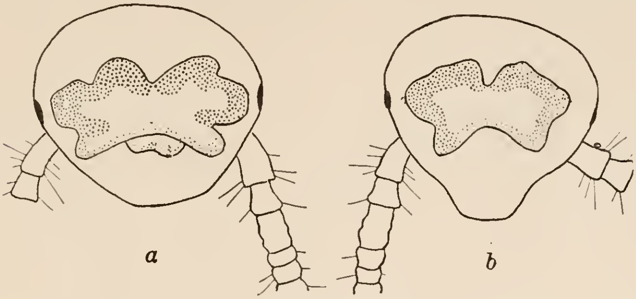

FIG. 3. *Termopsis angusticollis*, young nymphs 1.8 mm. long, with twelve antennal segments. *a*, head of reproductive nymph with large brain; *b*, head of soldier nymph with small brain. Oc. 6, obj. 16, reduced one third.

Slightly older nymphs of *Termopsis*—2.8–3 mm., with thirteen and fourteen antennal segments—have the heads differentiated in size so that the "small-headed," large-brained, reproductive forms are easily distinguishable from the "large-headed," small-brained, soldier nymphs.

Cryptotermes cavifrons Banks.—The egg of *Cryptotermes*, like those of the other Protermitidæ here described, is long slender and reniform, and measures 1.2–1.3 mm. in length (Fig. 1, *c*).

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[1] The worker caste is lacking in the genus *Termopsis*.

The newly hatched nymphs are 1.4–1.6 mm. long and have nine antennal segments, the third segment entire and bare. These nymphs are externally all alike, but are differentiated internally into the reproductive type with large brain, and large sex organs (Fig. 4, *a*), and the soldier[1] type with smaller brain and smaller sex organs (Fig. 4, *b*).

The peculiar truncated head and the characteristic form of the antennae of this species are recognizable, even in the newly hatched nymphs.

In nymphs which are about 2.4 mm. long the "small-headed" and "large-headed" types are clearly seen. This termite, like *Calotermes* n. sp., increases the antennal segments slowly, for nine segments still occur in this older phase, although the third segment is subdivided.

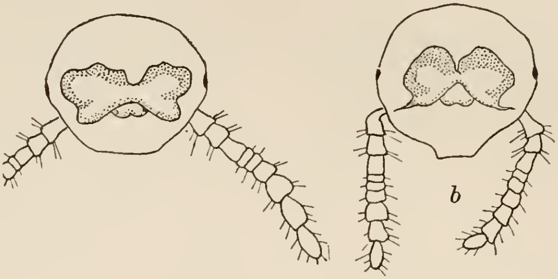

FIG. 4. *Cryptotermes cavifrons*, newly hatched nymphs. *A*, head of reproductive nymph with large brain; *b*, head of soldier nymph with small brain. Oc. 6, obj. 16, reduced one third.

Neotermes castaneus Burmeister.—The egg of *Neotermes*, fig. 5, *c*, is large, measuring 1.6 mm. in length. In form it is slender and reniform like the eggs of other Protermitidae.

The youngest nymphs in my material, which is not very abundant, are 2 mm. long and have ten antennal segments. Since the majority of the termites here described have nine antennal segments when hatched and are only from 0.2–0.3 mm. longer than the egg from which they have emerged, I believe that these nymphs are very slightly past the age of hatching. An examination of the antennae gives further basis for this opinion, fig.

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[1] The worker caste is lacking in the genus *Cryptotermes*.

5, *a*, *b*. The fourth antennal segment, which in growing individuals is always the youngest, is short and bears very minute hairs, and in one specimen bears hairs on one side only, indications that this segment has been very recently cut off from the third, probably since the time of hatching.

Externally these young nymphs of *Neotermes* are still all alike, for no differentiation in the size of the heads can be observed, but internally they are differentiated into the two types of nymph found in the other Protermitidae, namely: the reproductive or fertile type, with large brain, and large sex organs, and the soldier or sterile type, with small brain and small sex organs. The heads of these two types are shown in fig. 5, *a*, *b*.

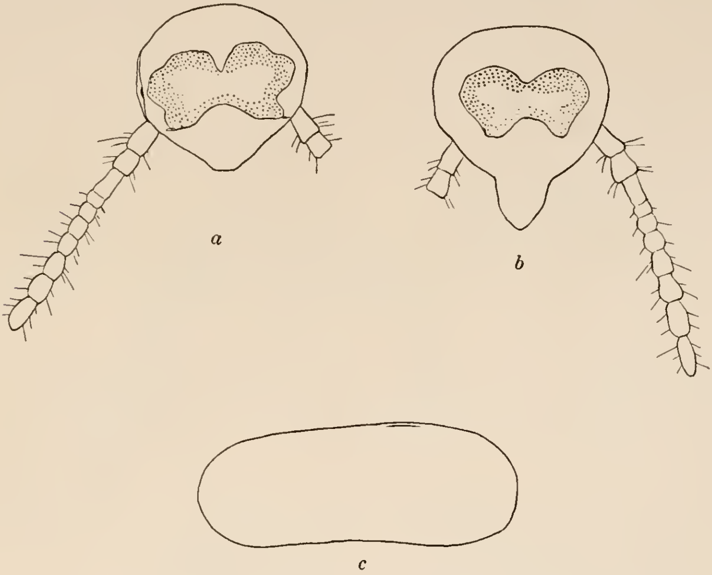

FIG. 5. *Neotermes eastaneus*, *a*, head of young reproductive nymph, 1.8 mm. long; *b*, head of young soldier nymph, 1.8 mm. long; *c*, egg. *a*, *b*, Leitz oc. 1, obj. 3; *c*, Leitz oc. 4, obj. 1; reduced one third.

Nymphs 2.6 mm. in length and with eleven antennal segments have the heads differentiated into the small-headed reproductive type with large brain, and the large-headed soldier type with small brain.

All four genera of Protermitidae are therefore alike in possessing two types of nymphs at the time of hatching.

MESOTERMITIDÆ.

Arrhinotermes simplex Hagen.—The eggs of the Mesotermitidæ are considerably smaller than those of the Protermitidæ. The eggs of *Arrhinotermes simplex* measure from 0.8 to 1 mm. in length and are slender and reniform (Fig. 1, *d*).

The newly hatched nymphs are about 1.2 mm. long, and have nine antennal segments, the third segment grooved and subdivided into two parts.

The two types of nymphs: (*a*) reproductive nymphs, with large brain and large sex organs (Fig. 6, *a*, *c*), and (*b*) worker-soldier nymphs, with small brain and small sex organs (Fig. 6, *b*, *d*), are likewise present in this genus at the time of hatching; and with an increase of the body length to 2 mm., the "small-headed" and "large-headed" nymphs are differentiated. There is a striking resemblance between the young of this genus and the related genus *Reticulitermes*.

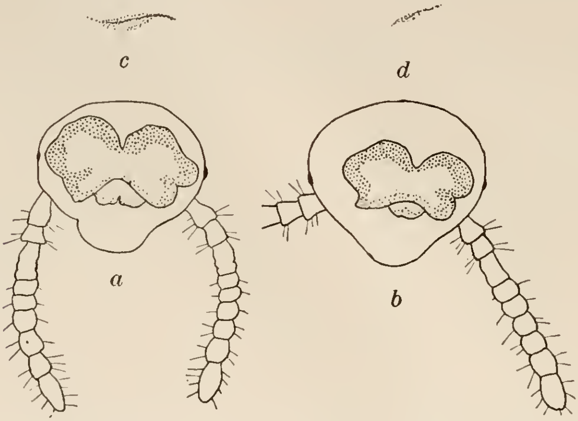

FIG. 6. *Arrhinotermes simplex*, newly hatched nymphs. *a*, head of reproductive nymph; *b*, head of worker-soldier nymph; *c*, ovary of reproductive nymph; *d*, ovary of worker-soldier nymph. Oc. 6, obj. 16, reduced one third.

Reticulitermes.

R. flavipes Kollar.[1] The egg measures from 0.68 to 0.7 mm. (Fig. 1, *e*). The newly hatched nymphs are 1.1 mm. long, with nine antennal segments, the third segment grooved, and are of

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[1] For a fuller description of the development of *R. flavipes*, see Thompson (1917).

the two types, reproductive nymphs and worker-soldier nymphs. Nymphs 2 mm. long with twelve antennal segments are differentiated into "small-headed" and "large-headed" types.

R. virginicus Banks.—The newly hatched nymphs of *R. virginicus* are 1 mm. long and have nine antennal segments, the third grooved. They are differentiated into the two types of large-brained reproductive forms and small-brained sterile forms. The further development of this species, so far as I have followed it, is very similar to and probably identical with that of *R. flavipes*.

R. n. sp. Banks.—The young nymphs of a new species of *Reticulitermes* not yet described have been given me by Mr. T. E. Snyder. Examination of this species shows that the conditions at the time of hatching are similar to those in *R. flavipes* and in *R. virginicus*. It seems more than probable therefore that this differentiation of the newly hatched nymphs into two types is common to all species of *Reticulitermes*.

METATERMITIDÆ.

Anoplotermes fumosus Müller.—The eggs of the Metatermitidæ are smaller than either of the other families of termites. The eggs of *A. fumosus* measure from 0.56 to 0.60 mm. in length (Fig. 1, *f*).

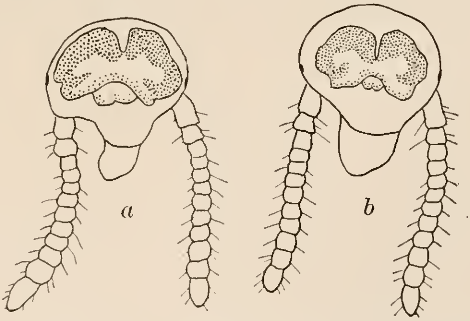

FIG. 7. *Anoplotermes fumosus*, young nymphs 1 mm. long, with twelve antennal segments. *a*, head of reproductive nymph; *b*, head of worker-soldier nymph. Oc. 6, obj. 16, reduced one third.

The youngest specimens of *Anoplotermes fumosus* that I have examined are in an early phase of development, but, on account of their size and the number of antennal segments, I do not think that they are the newly hatched forms. These specimens measure from 1 to 1.2 mm. in length, and have twelve antennal segments, the third segment being deeply grooved or subdivided. Like the very young nymphs of the other termite genera described above, these young nymphs of *A. fumosus* are externally all alike but are differentiated internally into (*a*) the reproductive nymphs, with large brain and large sex organs (Fig. 7, *a*), and (*b*) the worker-soldier nymphs, with small brain and small sex organs (Fig. 7, *b*). An unusually long slender labrum is present in both types of nymphs, and is characteristic of the three genera of this family here described.

The older nymphs of *A. fumosus*, about 2 mm. long, or less, are differentiated into the "small-headed," large-brained, reproductive type, and the "large-headed," small-brained worker-soldier type. My material was not very abundant, but was sufficient to show that the early development of *A. fumosus* is similar to that of the other termites here described.

Amitermes tubiformans Buckley.—The eggs of *A. tubiformans* measure from 0.56 to 0.64 mm. in length (Fig. 1, *g*).

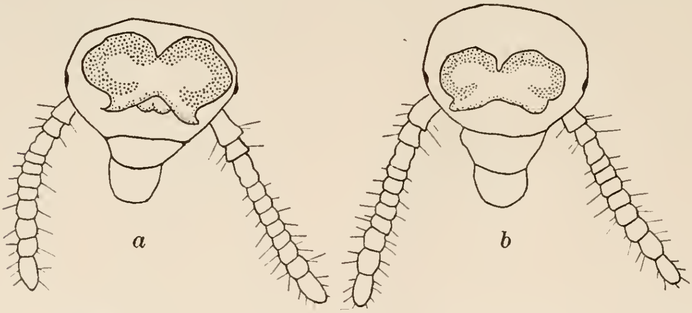

FIG. 8. *Amitermes tubiformans*, newly hatched nymphs. *a*, head of reproductive nymph; *b*, head of worker nymph. Oc. 6, obj. 16, reduced one third.

The youngest nymphs examined are from 0.95 to 1. mm. long, and have antennæ with eleven segments, the third segment bare, but subdivided into two parts. One of these nymphs had a piece of the egg shell still adhering to the abdomen, so that they are evidently the newly hatched forms.

The newly hatched nymphs of *A. tubiformans* are externally all alike, but are differentiated internally into (*a*) reproductive nymphs with large brain and large sex organs (Fig. 8, *a*), and (*b*) worker[1] nymphs, with small brain and small sex organs (Fig. 8, *b*). The long labrum is prominent in the nymphs of both types.

Nymphs 1.6 –1.8mm. long, with twelve antennal segments, are differentiated into the "small-headed" and "large-headed" types.

Eutermes.

E. morio Latreille.—The eggs of this species of *Eutermes* measure from 0.68 to 0.72 mm. in length and are slender and reniform. The newly hatched nymphs are 1–1.2 mm. long; they have eleven antennal segments, and they consist of the large-brained reproductive type and the small-brained worker-soldier type.

E. sanchezi Holmgren.—I have not seen the eggs of this species but the newly hatched nymphs are very similar to those of *E. morio*. They are 1.2 mm. long, with eleven antennal segments, and the two types of reproductive and worker-soldier nymphs are present.

The newly hatched nymphs of *E. morio* and *E. sanchezi* are almost identical in structure with those of *E. pilifrons* and are therefore not figured; their later development has not been studied, but there is no reason to believe that the three species, which are alike in their early development, should differ in the later phases.

E. pilifrons Holmgren.—The eggs of *E. pilifrons* measure from 0.64 to 0.72 mm., and are slender and reniform (Fig. 1, *h*).

This species was formerly described as *E. rippertii* (?) by Knower, and I am indebted to Dr. Knower for my very abundant material. Many of the nymphs have actually been dissected out from their egg shells, so that there is absolutely no uncertainty as to the structure or the size of the newly hatched forms.

The newly hatched nymphs of *E. pilifrons* measure from 0.8 to 0.9 mm., the difference in length being due to whether the nymphs are still in the curved embryonic position or have straightened out. The number of antennal segments is eleven,

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[1] The soldier caste is lacking in the genus *Amitermes*.

with the third segment grooved and subdivided into two parts, so that twelve and thirteen segments will be formed soon after hatching. It is interesting to note the progressive development of the termite order in as small a detail as the number of segments in the antennæ at the time of hatching. The Protermitidæ have nine antennal segments, with the third segment entire; in the intermediate Mesotermitidæ the number is still nine, but the third segment is grooved or subdivided into two parts; in the most highly developed Metatermitidæ the number has increased to eleven, with the third segment subdivided.

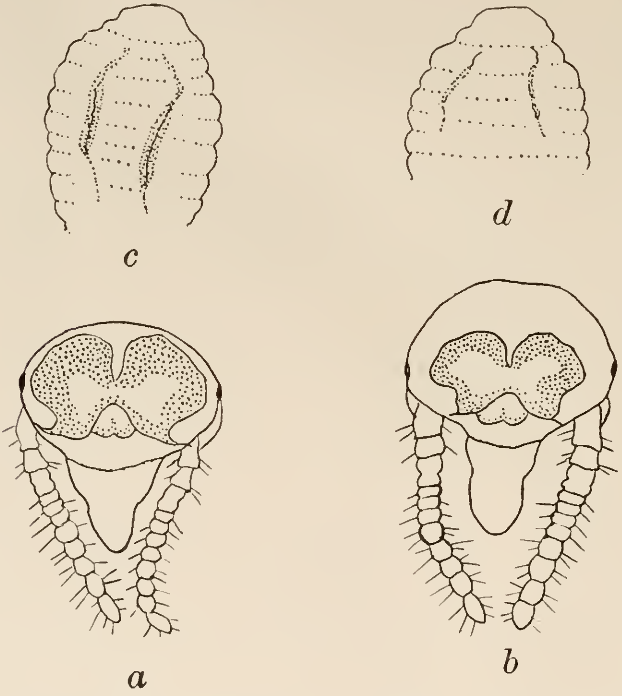

Fig. 9. *Eutermes pilifrons*, newly hatched nymphs. *a*, head of reproductive nymph; *b*, head of worker-soldier nymph; *c*, ovaries of reproductive nymph; *d*, ovaries of worker-soldier nymph. Oc. 6, obj. 16, reduced one third.

E. pilifrons, like all the other termites described in this paper, has the two types of newly hatched nymphs which are alike in external structure:—the reproductive nymphs, with a large brain and large sex organs (Fig. 9, *a*, *c*), and the worker-soldier nymphs, with a smaller brain and smaller sex organs (Fig. 9, *b*, *d*). The difference between the bodies of fixed specimens of the two types is more marked in this termite than in any other that I have examined; the body of the sterile worker-soldier individuals is a very clear and transparent glistening white, while that of the reproductive individuals is a dull opaque creamy white. A long slender labrum is noticeable in all nymphs of both types.

No appreciable difference in the size of the heads is noted until the nymphs have attained a length of about 2 mm., and have twelve antennal segments. In this phase the "small-headed" reproductive forms with large brain and creamy white body are easily distinguishable with a hand lens from the "large-headed" worker-soldier forms with small brain and transparent glistening white body.

In worker-soldier individuals about 2 mm. long and with twelve antennal segments there is as yet no external differentiation between the two sterile castes, but an internal differentiation has already begun and may be observed in whole mounts of stained individuals as well as in sections. The future soldiers are distinguishable by the presence of the larger frontal gland which appears, in frontal mounts of the head, as a small dense spot posterior to the brain; in whole mounts of the head of the future worker no such spot is visible. After examining the stained specimens in cedar oil to separate the future soldiers from the future workers, the two kinds of individuals were embedded and sectioned. In the soldier nymphs a large, although embryonic, frontal gland opens to the exterior on the frontal surface of the head. This gland was more than three times the size of the vestigial gland found in the worker nymphs.

The soldier caste of *E. pilifrons* is, therefore, not differentiated by external characters at the time of hatching, but arises later in the development, being first manifested in individuals about 2 mm. long with twelve antennal segments. The worker caste is differentiated at the same time, and the two castes may be recognized by the size and structure of the respective frontal glands; although no external differentiations are yet present in either caste.

The differentiation of the worker-soldier nymphs of *E. pilifrons* into the worker and the soldier is nearly parallel with the development of these two castes from the worker-soldier form in the genus *Reticulitermes*, Thompson (1917, pp. 123–125); the chief difference being the age of the respective nymphs, the differentiation being visible in *E. pilifrons* in nymphs 2 mm. long, while in *R. flavipes* it was first observed in nymphs 3.75 mm. long, although, from the maturity of the frontal gland, it could probably be seen in an earlier phase.

My work upon this species of *Eutermes* is in perfect accord with the work of Knower (1894), who noted the absence of young soldier nymphs in the colonies of *E. rippertii* (?) = *E. pilifrons* Holm., and who later saw a young soldier of this species emerge by molting from a worker-like skin. The mandibles on the skin were large, as in *Eutermes* workers, while those of the emerging soldier (nasutus) were small, like those of the adult nasuti. The head of this nasutus was light in color and not yet fully elongated.[1]

Bugnion (1912) states that the soldier (nasutus) of *E. lacustris* is differentiated at the time of its emergence from the egg, and is distinguished from the other newly hatched nymphs by a long frontal process or horn, and a frontal gland with an excretory canal. His description is as follows:

"Larve de soldat, longue de 1.32 mm, venant d'éclore (Fig. 12). Cette forme est particulièrement intéressante parce qu'elle montre une petite corne implantée au dessus du front. Elle donne ainsi la preuve que la caste 'soldat' se différencie déjà dans l'oeuf. La corne, tres courte, ne dépasse pas le niveau des pièces buccales. On voit aussi, par transparence, l'ampoule céphalique entourée de muscles et, à la base de la corne, le canal excréteur."

Now, although I am fully in accord with the view of Bugnion that the castes of termites are not produced by food or other external influences, and that they are due to intrinsic deep-seated causes, I am inclined to believe, for several reasons which follow, that Bugnion's account of the newly hatched soldier (nasutus) nymph of *E. lacustris* may be capable of a different interpretation.

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[1] Snyder (1915) has also described the origin of a soldier nymph from a worker-like form in *Reticulitermes flavipes* and *R. virginicus*. The nymphs were found in the quiescent stage that precedes a molt, and the emergence of the young soldiers was observed. In this case, too, the mandibles of the emerging form differed from those on the cast skin.

First, the conditions among the newly hatched and the younger developing phases of the nine termite genera here described are practically similar, showing that throughout the entire order of termites the early development is remarkably constant.

Second, I have examined the newly hatched and the developing nymphs of three species of *Reticulitermes*—*R. flavipes*, *R. virginicus* and *R.* n. sp.—and also of three species of *Eutermes*—*E. pilifrons*, *E. morio* and *E. sanchezi*—and in each case the development of the three species is similar throughout the early phases and as far as I have followed it. It seems probable, therefore, that *E. lacustris* would follow the plan of development that is common not only to three other species of its genus and to three species of *Reticulitermes*, but to seven other genera.

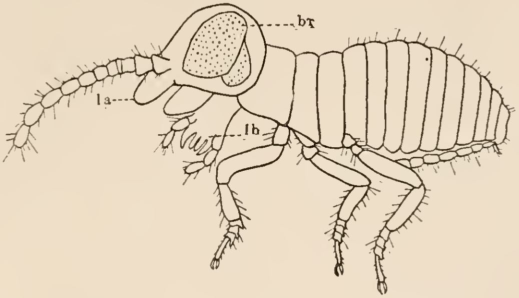

FIG. 10. *Eutermes pilifrons*, newly hatched nymph, profile view. *la*, labrum; *lb*, labium; *br*, brain. Oc. 6, obj. 16, reduced one half.

Third, in all the newly hatched nymphs of *Eutermes*, whether *pilifrons*, *morio* or *sanchezi*, the labrum, as stated above, is long slender and conspicuous. In nymphs seen in profile the side view of this labrum is surprisingly like the frontal process or "corne frontale" of a nasutus. In studying the younger *Eutermes* nymphs in alcohol I remarked again and again the length of the labrum and its resemblance, especially in profile view, to the frontal process of a nasutus. This is also true of the other two genera of the Metatermitidæ here described, *Anoplotermes* and *Amitermes*.

In my Fig. 10, the profile view of a recently hatched *worker-soldier* nymph of *E. pilifrons*, the resemblance is very striking to Bugnion's figure 12 (1912), the profile view of the form which Bugnion has described as a newly hatched *soldier* nymph of *E. lacustris*. The long labrum, *la*, might have been taken for the "corne frontale," the dark stippled mass, *br*, which represents the brain as seen through the transparent skin of the head, might easily be mistaken for the frontal gland that is shown in Bugnion's figure in about the same position. The duct of the frontal gland figured by Bugnion might prove to be the esophagus, which passes forward as a slender tube beneath the brain. Furthermore, the frontal gland as figured by Bugnion in the newly hatched soldier (nasutus) of *E. lacustris* is much larger and more highly developed than in the older nymphs of *E. pilifrons* 2 mm. long, and is also larger and more developed than I have found it in any newly hatched termite nymphs that I have studied. As a rule this gland is not distinguishable at the time of hatching except in sections, and even then is extremely small and difficult to determine.

Fourth, Professor Bugnion's figure was drawn from an unstained specimen, mounted, as he states, in either water or weak formol, and studied as a transparent object. If after staining and sectioning the same results are obtained in the newly hatched nymphs of *E. lacustris* we should then have positive proof that development varies in the different species of a termite genus and that some castes may be fully differentiated at the time of hatching. But, until this proof is established, the writer is inclined to believe that development is a constant process among the different species of termites, and that it takes place as in the termites here described.

SUMMARY.

The termites described in this paper are:

PROTERMITIDÆ—
- *Termopsis angusticollis.*
- *Calotermes* n. sp.
- *Cryptotermes cavifrons.*
- *Neotermes castaneus.*

MESOTERMITIDÆ—
Arrhinotermes simplex.
Reticulitermes flavipes.
" *virginicus.*
" n. sp.
METATERMITIDÆ—
Anoplotermes fumosus.
Amitermes tubiformans.
Eutermes morio.
" *sanchezi*
" *pilifrons.*

1. The eggs of these termites are largest in the primitive forms, the Protermitidæ, measuring from 1.2 to 1.7 mm. in length, they are smaller in the Mesotermitidæ, from 0.68 to 1 mm. long; and are smallest in the Metatermitidæ, from 0.56 to 0.72 mm. long.

2. The newly hatched nymphs correspond in size to the eggs from which they have hatched.

3. The newly hatched nymphs are externally all alike, but they are differentiated by internal structural characters into *two* clearly defined types: (*a*) the *reproductive*, or *fertile forms*, with large brain and large sex organs, and usually a dense opaque body; and (*b*) the *worker-soldier*, or *sterile forms*, with small brain and small sex organs, and usually a clear transparent body.

4. At the time of hatching the antennæ of the Protermitidæ (*Calotermes* n. sp., *Cryptotermes cavifrons*, and probably also *Termopsis angusticollis* and *Neotermes castaneus*) have nine segments, with the third segment entire; those of the Mesotermitidæ (*Arrhinotermes simplex*, *Reticulitermes flavipes*, *R. virginicus* and *R.*, n. sp.) have nine segments with the third subdivided; those of the Metatermitidæ (*Amitermes tubiformans*, *Eutermes pilifrons*, *E. morio* and *E. sanchezi*, and probably also *Anoplotermes fumosus*) have eleven segments, the third subdivided.

5. Nymphs with a body length of about 2 mm., or 2.5 to 3 mm. in the larger genera, are differentiated into "small-headed" but large brained reproductive forms, and "large-headed" but small-brained worker-soldier forms.

6. The worker-soldier nymphs of *Eutermes pilifrons*, 2 mm.

long, with twelve antennal segments, and externally all alike, are distinguishable, after staining, into worker nymphs with a small vestigial frontal gland, and soldier nymphs with a large frontal gland.

7. The soldiers of *Eutermes pilifrons*, and also those of *E. morio* and *E. sanchezi*, are therefore not externally differentiated at the time of hatching, which, according to Bugnion, is the case in *E. lacustris*.

WELLESLEY, MASS.,
July, 1918.

BIBLIOGRAPHY.

Bugnion, E.

'12 *Eutermes lacustris* nov. sp. de Ceylan. Revue Suisse de zoologie, vol. 20.

'12b Obsérvations sur les termites. Différentiation des castes. Comp. Rend. soc. biol. Paris, I., t. 72.

Grassi, B., and Sandias, A.

'93–'94 Costituzione e sviluppo della società dei Termitidi. Atti Acad. Gioenia di sci. nat., Catania.

'96–'97 The Constitution and Development of the Society of Termites; Observations on their Habits; With Appendices on the Parasitic Protozoa and on the Embiidæ, translated by T. H. Blandford. Quart. Jour. Micros. Sci., vols. 39–40.

Knower, H. McE.

'94 Origin of the "Nasutus" (soldier) of *Eutermes*. Johns Hopkins Univ. Bull., vol. 13, pp. 58–59.

Snyder, T. E.

'15 Biology of the Termites of the Eastern United States with Preventive and Remedial Measures. U. S. Dept. Agr., Bur. Ent., Bull. no. 94.

Thompson. C. B.

'16 The Brain and the Frontal Gland of the Castes of the "White Ant," *Leucotermes flavipes* Kollar. Jour. Comp. Neurol., vol. 26.

'17 Origin of the Castes of the Common Termite, *Leucotermes flavipes* Kol. Jour. Morph., vol. 30.

RESEARCHES ON THE SEX-CHROMOSOMES OF PSYCHIDÆ (LEPIDOPTERA).

J. SEILER.

WITH ONE TEXT-FIGURE AND ONE PLATE.

The demonstration which I have given of the digametic condition of the female sex in Lepidoptera in the case of *Phragmatobia*,[1] gave a brilliant confirmation of the assumption made by genetic experimenters (Doncaster, Goldschmidt) that in butterflies the female sex must be digametic. In all other cases the digametic condition has been conclusively demonstrated only in the male, and as was to be expected, therefore, my results were doubted. For this reason I sought for a typical case in which the distribution of the sex-chromosomes could readily be demonstrated, and to work it out in such a manner as to leave no room for further doubt.

Such a typical case was found in certain species of Psychidæ. I was led to examine this group because the sex-ratios (which under certain conditions are quite atypical) gave reason to look for interesting cytological conditions; for it might be expected that in the parthenogenetic species, as is the case in *Angiostomum*, *Phylloxera*, etc., the preponderance or exclusive appearance of only one sex might be determined by the sex-chromosome mechanism. Unfortunately this point could not be demonstrated; on the other hand, the diœcious forms, *Talæporia tubulosa* and *Fumea casta*, were found to offer material suitable for my main purpose.

1. *Talæporia tubulosa* RETZ.

(*a*) *Maturation of the Egg.*—As the daughter-chromosomes of the first maturation-division separate, one chromosome remains at the middle of the spindle without showing at first any indication as to whether it will pass to the inner or the outer pole (Figs. 1–3). In Fig. 3 it will probably pass inwards, as may be inferred from its relation to the slightly developed spindle-thickening. As the daughter-chromosomes move towards the spindle-poles the X-chromosome is found to lag behind, in some cases towards the outer plate, in others towards the inner. In Fig. 4 it is seen wandering inwards, in 5 outwards, in 6 and 7 inwards, in 8 outwards. During the interkinesis it is only occasionally recognizable (Fig. 9). In the metaphase of the second division (Fig. 10) it has without exception overtaken the autosomes and thenceforwards shows precisely the behavior of an autosome. Only in exceptional cases does the X-chromosome move from the beginning at the same rate with the autosomes; in such cases, of course, it is not distinguishable in side-views of the spindle.

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[1] '13, '14, *Arch. Zellforsch.*, Vol. XIII.

In the Psychidæ, as in other cases (see Seiler, '14), chromatin is in some instances eliminated in large quantities during the first maturation-division, in others not at all.

The equatorial plate of the first maturation-division in the egg possesses 30 chromosomes, of which 29 are bivalent and one univalent. As is to be expected, the daughter-plates have different numbers of chromosomes. If the outer plate has 30 chromosomes the inner has 29, and *vice versa.* I have tried to obtain photographic proof of this, but the task is extremely difficult and tedious. Each egg contains but one spindle, and this must be so cut that the knife passes between the two daughter-plates without injuring them. Further, all the chromosomes (of course in both plates) must lie exactly in one plane and at right angles to the optical axis. Fig. 18 shows such an ideal plate with 29 chromosomes, and this photograph alone reproduces the size-relations of the chromosomes exactly as in nature. The sister-plate is not suitable for photographing. *All the plates photographed for this work are in themselves quite as clear and demonstrative* as Fig. 18. Since, however, the chromosomes did not always lie in the same plane, it was necessary to turn the fine adjustment somewhat during the exposure; hence the somewhat defective and in part ambiguous character of the figures. Figs. 11 and 12 are two sister-plates (in each case the first number designates the outer plate, the second number the inner), in the anaphase of the first division. Since the chromosomes undergo little or no change of relative position during the separation of the daughter-plates, the homologous pairs can be identified with considerable certainty. In Figs. 13 and 14, again showing two sister-plates, the X-chromosome lies centrally.

Table I., which follows, gives a summary of selected cases in which both daughter-plates lie entirely in the same section, and which in other respects fulfill all the conditions of demonstrative clearness. Table II. summarizes the number of observed cases in which the X-chromosome as seen in lateral views of the spindle, is passing towards the outer or the inner pole. The result permits us to predict the primary sex-ratio to be expected in *Talæporia*. Those X-chromosomes that pass outwards enter the polar body, those that pass inwards divide equally in the second maturation-division and enter the female pro-nucleus.

TABLE I.

Locality.	No.	No. of Preparation.	No. of Chromosomes.	
			Outer Plate.	Inner Plate.
Breslau	1	1.14	30	29
"	2	1.10	29	30
"	3	26.6	30	
"	4	51.2	30	
Tornow	5	122.5	30	29
"	6	125.9	29	
"	7	136.4	30	29
"	8	136.1		30
"	9	136.9		29
"	10	136.10	29	
"	11	136.13	29	
"	12	136.17	29	30
"	13	137.3	30	29
"	14	138.5	30	29
"	15	138.7		29
"	16	138.8	29	
"	17	138.12		30
"	18	138.11		29
"	19	138.16	30	
"	20	139.11		29
"	21	139.13		29
"	22	140.6	29	30
"	23	140.10	29	
"	24	140.5	29	
"	25	149.7	29	30
"	26	150.20	30	
"	27	151.2		30
"	28	151.3	29	30
"	29	152.2	30	29
"	30	153.14	30	
"	31	153.16		30
"	32	155.1	30	
"	33	6.11	29	29
"	34	140.18	29	29

(*b*) *Maturation of the Sperm.*—In the spermatogenesis no sex-chromosomes can be distinguished. The equatorial plates of the first maturation-division show 30 chromosomes (Figs. 26, 27) and those of the second division the same (Fig. 28).

TABLE II.

Locality.	The X-Chromosome Passes		Total.	Sex Ratio.	
	Outwards.	Inwards.		Female.	Male.
Breslau........	73	44	117	1.66	1
Tornow........	61	45	106	1.35	1
	134	89	223	1.50	1

(*c*) *The Somatic Chromosome-number.*—Since two classes of eggs are formed, having respectively 29 and 30 chromosomes, we may expect to find two kinds of embryos, one having 59 chromosomes, the other 60. The actual conditions correspond with this expectation. In the stage of blastoderm-formation it is possible to count the somatic chromosome-number with absolute certainty. Figs. 15 and 16 show 60 chromosomes, though not so clearly as the actual object. Table III. gives a summary of the observed cases. Only such embryos were recorded as showed at least 2 (often 4 to 6) perfectly clear equatorial plates, each of which lay entirely in a single section and in the middle of its thickness, and in which no doubt could exist concerning the limits of a single chromosome.

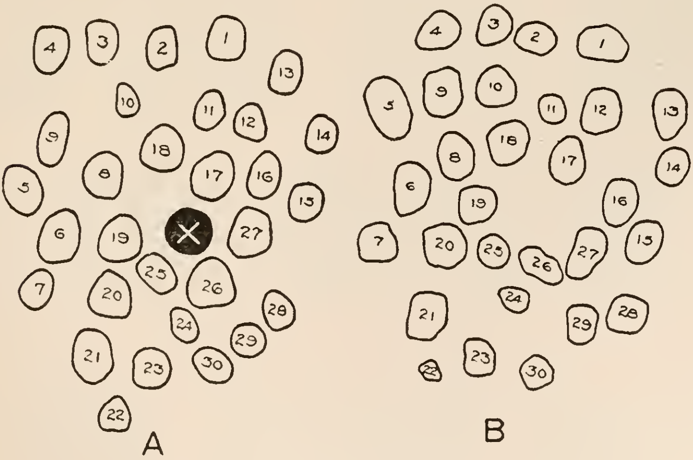
TABLE III.

Locality.	No. of Embryos.	Chromosomes.
Tornow....................	30	59
"	25	60
"	4	58

As the table shows, a third sort of embryo could be demonstrated possessing 58 chromosomes. This determination can not possibly rest on an error, for in the first embryo seven perfectly clear plates all showed 58 chromosomes, in the second 6, in the third 5, and in fourth 2. Such embryos would probably produce but one kind of egg, containing 29 chromosomes. In point of fact, I have two perfect pairs of daughter-plates, each with 29 chromosomes (see Table I., nos. 33, 34). No X-chromosome is present in these cases. If we leave these out of account there remain 35 females and 25 males, a sex-ratio of 1.36: 1 in favor of the female, that is to say, exactly the ratio to be expected from observations on the maturation-phenomena in the Tornow race. Possibly the embryos with 58 chromosomes may have arisen by the development of unfertilized eggs.

2. *Fumea casta* Pall.

The equatorial plate of the first maturation-division in the egg shows 31 chromosomes. One of these must be univalent, for the daughter-plates show in some cases 31 chromosomes, in others 30. As in *Talæporia*, an X-chromosome is present but strange to say it fails to lag behind the autosomes and passes together with them to one spindle-pole. Fig. 23, from a perfect plate with 30 chromosomes, clearly shows the natural size-relations. Figs. 19 and 20, 21 and 22, 24 and 25 are three pairs of daughter-plates, from the interkinesis, in which the chromosomes are perfectly clear and well-separated; but unfortunately the plates lay obliquely, so that the photographs seem to show certain fusions which do not really exist. Text-figure 1 shows the same



Text Fig. 1.

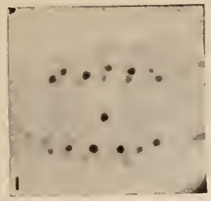
plates as Photos. 19–20, reproduced as accurately as possible in respect to the size-relations and relative positions, with an attempt to identify the homologous chromosomes. The X-chromosome happens, quite by accident, to be in all three cases in the outer plate; quite as often it is found in the inner one.

The embryos have either 61 or 62 chromosomes.

January, 1917.

EXPLANATION OF PLATE.

None of the photographs retouched. Enlargement 2,000, excepting Fig. 10, which is 1,000.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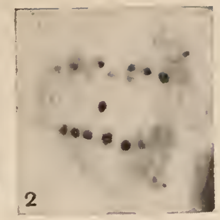
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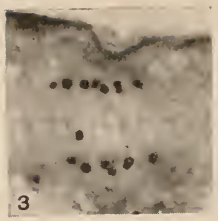
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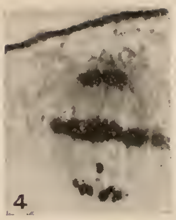
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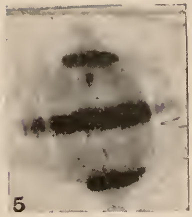
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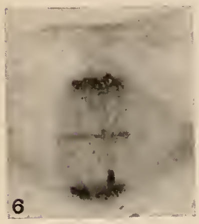
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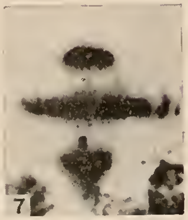
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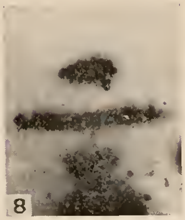
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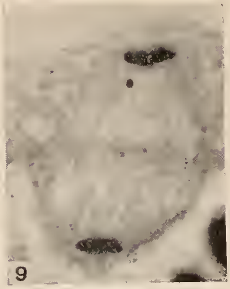
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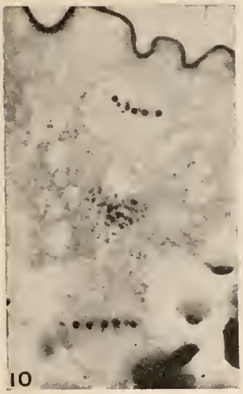
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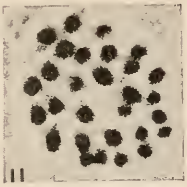
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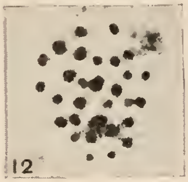
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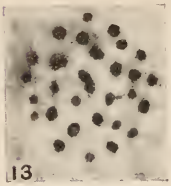
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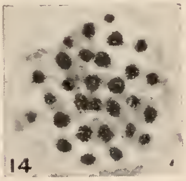
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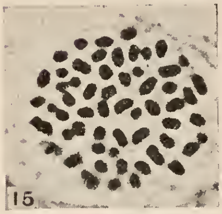
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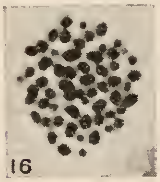
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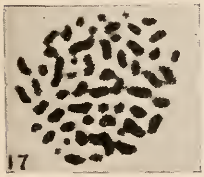
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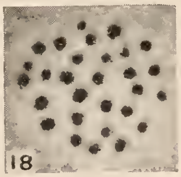
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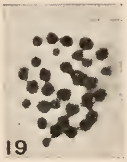
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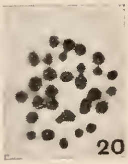
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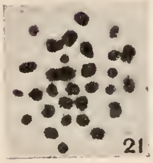
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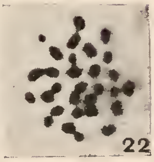
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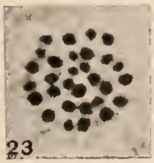
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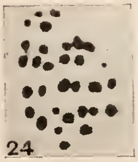
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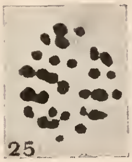
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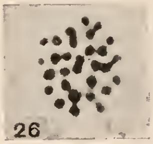
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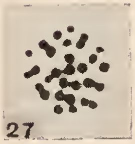
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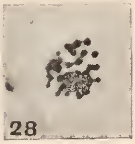
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J. SEILER

THE RELATION OF THE PITUITARY AND THYROID GLANDS OF BUFO AND RANA TO IODINE AND METAMORPHOSIS.[1]

BENNET M. ALLEN.

UNIVERSITY OF KANSAS.

In the spring of 1918, Mr. Swingle and Mr. Etzen, working in our laboratory, discovered the fact that iodine administered in loose combination with flour brings about precocious metamorphosis in tadpoles after the same manner as the feeding of thyroid gland preparations previously demonstrated by Gudernatsch, '12. It at once occurred to the writer that iodine administration would afford a means of testing out the rôle of these two glands in metamorphosis. My plan was to administer iodine in this manner to tadpoles deprived of their thyroid glands, to those deprived of the pituitary gland and to those deprived of both. Since the thyroid gland has always been so completely identified with iodine in the animal body, the writer suggested that Mr. Swingle make it a part of his problem to feed iodine to thyroidless tadpoles. This he did with the result that he demonstrated the fact that the administration of iodine by feeding produces metamorphosis just as truly in thyroidless tadpoles as in normal tadpoles. It was shown by the work of Adler, '14, Smith, '16, and Allen, '16, that the removal of the pituitary gland of tadpoles has a marked effect upon the thyroid gland in that the colloid of the latter is of looser texture and smaller amount than normal. Associated with this is the fact that pituitaryless tadpoles do not undergo metamorphosis. A pituitaryless tadpole of *Rana pipiens* has been kept by the writer for two years without undergoing metamorphosis although it has now grown beyond the size normally attained by tadpoles of this species. These facts have led the writer to form the hypothesis that the pituitary gland may play an active rôle in metamorphosis, and that the thyroid gland would play the part of a storage organ. This view was expressed in an earlier paper

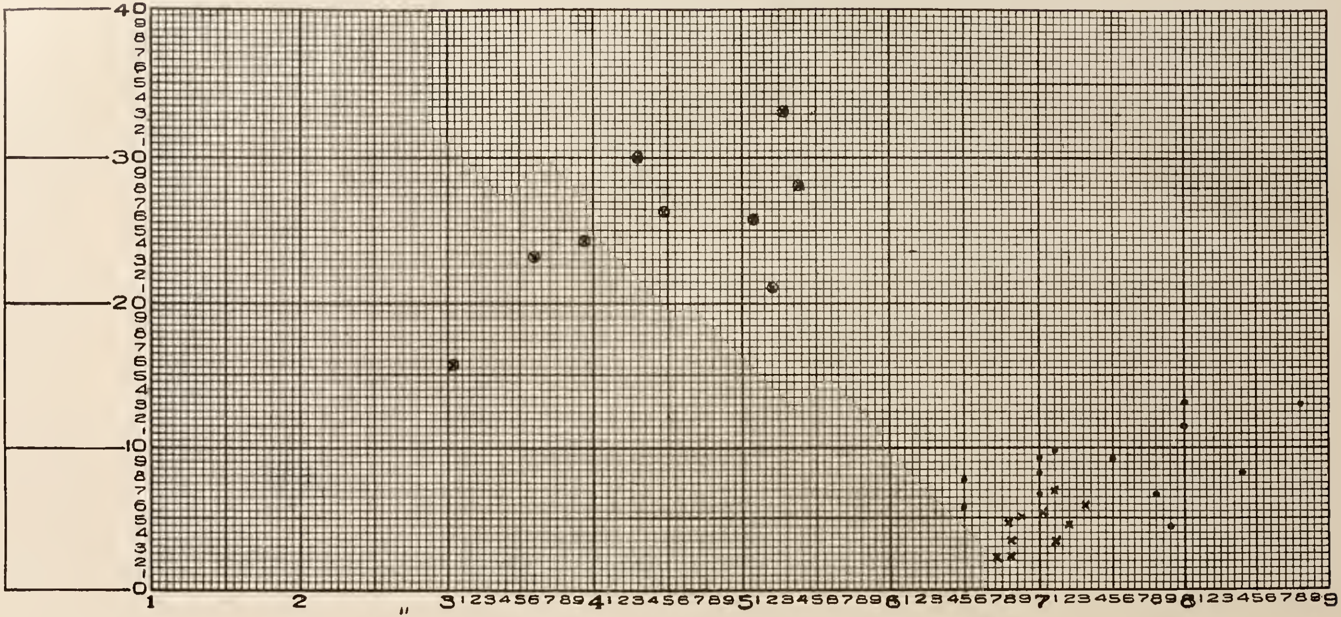

FIG. 1. Showing changes of body length and leg length of pituitaryless *Bufo* tadpoles to which iodine was fed. Abscissæ indicate body length in millimeters. Ordinates indicate hind leg length in millimeters. Dots indicate normal control tadpoles. Crosses indicate pituitaryless tadpoles at beginning of iodine feeding. Crosses in circles indicate pituitaryless tadpoles at end of iodine feeding.

(Allen, '17). Swingle's experiments showed that the administration of iodine alone without the mediation of the thyroid gland is able to produce metamorphosis. The peculiar correlation between the thyroid and pituitary glands made it seem highly desirable to carry on experiments in feeding iodine to tadpoles deprived of the pituitary gland and also to those deprived of both the thyroid and the pituitary glands.

The results of this experiment are so definite that they seem well worth recording. A large amount of caution is essential in work of this kind because it has been found that the characteristic color change described by Smith and Allen is not an altogether reliable test of the successful removal of the pituitary gland nor is the failure to metamorphose always to be relied upon as a test of success in the removal of either the pituitary or the thyroid gland. In certain cases, the color change has taken place in spite of the presence of a small remnant of the pituitary gland, and very greatly delayed metamorphosis has been found to take place in tadpoles in which a small fragment of the thyroid gland remained after the operation for removal. Notes along these lines have been published by the writer. For these reasons, it seemed best to make an intensive study of a limited number of specimens rather than to make a necessarily cursory study of a larger number. If the thyroid gland had been removed, the region where it should occur was sectioned and painstakingly studied in order to determine whether the operation had been successful. In the majority of cases where the pituitary gland had been removed, the region involved was likewise sectioned and examined for vestiges of the gland. The work was in this manner checked up to such a degree that the results are offered with full confidence of their accuracy. In these experiments, iodine was mixed with flour in the proportion of 1 to 100. Water was then added to make a creamy paste. This was then dried and the flakes were fed to the tadpoles. In the first series of experiments, *Bufo* tadpoles deprived of their pituitary glands were fed iodine for from ten to twenty-one days. This produced most striking results as shown in Tables I. and II. In this experiment, the tadpoles were kept in the same receptacle and as a consequence it was not possible to follow the changes in individuals but we must consider mass results. There is a marked decrease in the size of the body. This is out of all proportion to the reduction in body size during normal metamorphosis. There is marked decrease in the length of the tail amounting to complete disappearance in one specimen which was unfortunately not preserved owing to the drying up of the shallow water in which it was necessary to keep it. In ten of the fourteen cases here recorded, both fore limbs had broken through the skin and in three of them the left one alone had appeared.

The difficulties of rearing these larvæ to complete metamorphosis are considerable, but not insurmountable. The small size of the larvæ is no doubt one factor. The sudden marked shrinkage of the body is a decidedly abnormal feature. These results are quite comparable to those attained by Swingle in normal and in thyroidless tadpoles. The removal of the pituitary gland in *Bufo* causes it to assume a light buff color in place of the familiar intense black. This color change was in no wise modified by the subsequent iodine feeding. Fig. 1 shows in graphic fashion the nature of these changes as regards the typical feature of hind leg length. The abscissæ represent the length of body while the ordinates represent the length of the hind legs. The location of any point serves to indicate the length of body by its horizontal location and the length of the hind legs by its height above

Table I.

Bufo Pituitaryless Tadpoles Before Feeding Iodine.			No.	Iodine Feeding Begun May 31, 1918, After Feeding Iodine.					Days Fed.
Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.		Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Fore Leg Out.	
14.8	6.9	.50	1.	9.3	3.93	5.37	2.44	L.	21
14.6	7.2	.46	2.	5.9	3.60	2.34	2.34	R. L.	21
12.6	6.8	.22	3.	8.2	4.29	3.91	3.00	L.	28
13.0	6.7	.32	4.	9.2	4.49	4.71	2.67	R. L.	18
14.2	7.1	.76	5.	9.6	5.08	4.52	2.57	R. L.	18
16.1	7.3	.59	6.	7.5	3.03	4.47	1.52	R. L.	17
14.5	6.8	.46	7.	9.9	5.38	4.52	2.80	R. L.	18
14.4	7.1	.34	8.	11.3	5.28	6.02	3.30	R. L.	18
13.9	6.8	.31	9.	11.6	5.20	6.40	2.10	....	10
15.1	7.0	.54							
14.3	6.97	.44		9.18	4.67	4.69	2.59		

the base line. Dots are used to designate control, normal tadpoles; crosses designate the pituitaryless tadpoles just before the administration of iodine while crosses within circles indicate the pituitaryless tadpoles at the end of the period of iodine feeding. It appears that the pituitaryless tadpoles at the beginning of the experiment are at this stage already commencing to undergo a retardation in hind leg growth as compared with the normal controls. In the iodine fed, pituitaryless tadpoles the reduction in body length together with an actual and relative increase in leg length are clearly evident.

The next series of experiments involved feeding iodine in the same manner to tadpoles from which both the pituitary and thyroid glands had been removed. In this case, tadpoles were kept separated in different dishes so that it was possible to follow the growth of each individual tadpole. Unfortunately, all died within ten days. This may have been partly due to the excessively warm weather at the time. Because of the short period of the experiment, the results are not so striking as one might desire but they point in the same direction as the other experiments and are quite significant. Table III. gives the results and needs no further explanation. Table IV. gives the dimensions of *Bufo* tadpoles deprived of both thyroid and pituitary glands but maintained under normal food conditions. A comparison of the tables shows that these do not have hind legs of length at all comparable with those that were fed iodine, even though the

TABLE II.

Bufo Pituitaryless Tadpoles Before Feeding Iodine.				No.	Iodine Feeding Begun June 18, After Feeding Iodine.					Days Fed.
Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.		Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Fore Leg Out.	
20.6	9.8	10.8	.93	10.	10.7	6.4	4.30	3.17	R. L.	18
18.8	9.7	9.1	.88	11.	9.1	4.55	4.55	2.05	L.	18
18.5	8.8	9.7	.51	12.	10.3	4.82	5.48	2.41	R. L.	23
15.6	8.4	7.2	.53	13.	8.2	5.15	3.05	2.57	R. L.	R. L.
14.8	7.3	7.5	.38		9.6	5.23	4.34	2.55		
18.8	8.7	10.1	1.37	Iodine Feeding Begun June 3.						
17.8	8.8	9.1	.77	14.	9.3	6.34	2.96	3.60	R. L.	15

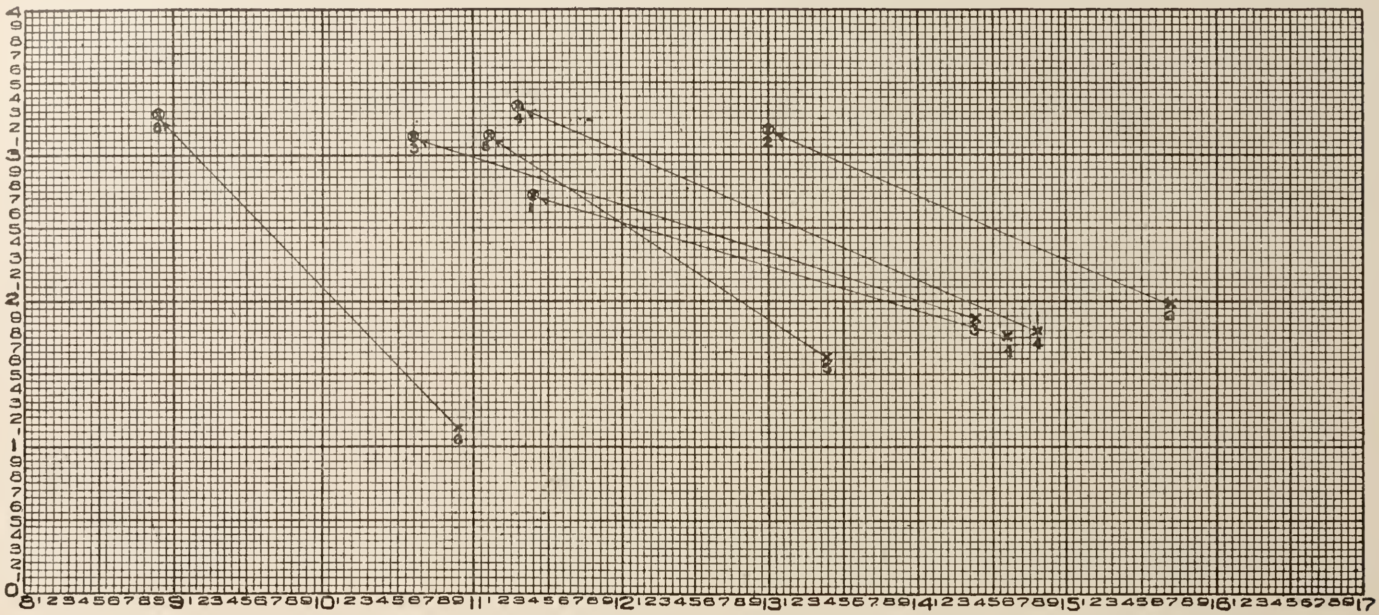

FIG. 2. Showing modifications of body length and leg length of pituitary-thyroidless tadpoles of *Bufo* to which iodine was fed. Abscissæ indicate body length in millimeters. Ordinates indicate hind leg length in millimeters. Arrows connect the points indicating any given individual at the beginning and the end of the experiment. The numbers of the points correspond to the numbers of Table III.

TABLE III.

Bufo PITUITARY-THYROIDLESS TADPOLES.

No.	Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Sucess of Operation.	Length of Feeding.
1.	33.1	14.6	18.5	1.75	No pituitary. No thyroid.	7 days.
2.	33.4	15.7	17.7	1.95	No pituitary. No htyroid.	10 days.
3.	32.5	14.4	18.1	1.91	No pituitary. No thyroid.	10 days.
4.	32.6	14.7	17.9	1.78	No pituitary. No thyroid.	10 days.
5.	29.1	13.4	15.7	1.62	Minute vestige of pituitary. No thyroid.	10 days.
6.	25.8	10.9	14.9	1.12	No pituitary. No thyroid.	10 days.
	31.2	13.9	15.5	1.69		

IODINE FEEDING BEGUN JULY 8.

Condition at End of Experiment.

No.	Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.
1.	26.2	11.4	14.8	2.71
2.	25.1	13.0	12.1	3.17
3.	26.1	10.6	15.5	3.13
4.	27.2	11.3	15.9	3.33
5.	27.9	11.1	16.8	3.14
6.	18.5	8.9	9.6	3.27
	25.2	11.0	14.3	3.12

tadpoles represented in Table IV. were killed about two and one half months later and were then not only of larger size but older as well. Data are accumulating to show that the hind limbs continue to grow proportionately longer in older specimens than in younger ones of equal size. This seems to be generally true of thyroidless, of pituitaryless and of thyroid-pituitaryless tadpoles. More data upon this point must be gathered before a final conclusion can be reached because there are decided individual differences in the rate of limb growth in normal and operated tadpoles under apparently identical food conditions. In any case, a comparison of Tables III. and IV. will serve to answer any possible objections upon this score.

Fig. 2 shows the modifications of body length and leg length indicated in Table III. The numbers of the points apply to the same individuals shown in the table. The arrow lines connect up points showing the dimensions of each specimen at the beginning and at the end of the feeding.

Table IV.

Bufo Pituitary-thyroidless tadpoles.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Date Killed.
1.	20.4	9.8	1.75 K.	Sept. 24.
2.	23.0	10.9	2.14 K.	Sept. 24.
3.	24.1	11.3	2.54 K.	Sept. 26.
4.	26.1	11.4	2.41 K.	Sept. 24.
5.	25.4	13.2	1.91 K.	Oct. 31.
	23.8	11.2	2.15	

Table V. shows the results of feeding iodine to *Rana pipiens* tadpoles from which both the thyroid and pituitary glands had been removed. The experiment was begun with five specimens, two of which were not preserved. These five tadpoles at the beginning of iodine feeding had body lengths ranging from 10.3 mm. to 15.2 mm. and the hind legs ranged in length from .28 mm. in the smallest to .80 mm. in the largest. It is probable that no. 1 in Table V. represents the largest and no. 3, the smallest but it is not possible to be certain upon this point because the tadpoles were all kept together in the same aquarium. In any case, the growth of the hind legs is remarkable and it will be noted that the left fore limb has appeared in nos. 2 and 3. The heads of these three tadpoles were sectioned and the series carefully studied in order to detect any remnants ever so small of thyroid or pituitary glands. Not the slightest vestiges of these

Table V.

Rana pipiens with Pituitary and Thyroid Glands Removed.

Fed Iodine from June 30 to July 25.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Fore Limbs.	Remarks.
1.	23.5	10.4	6.04	None	No thyroid. No pituitary.
2.	19.8	9.1	4.16	Left free	No thyroid. No pituitary.
3.	17.1	7.2	2.77	Left free	No thyroid. No pituitary.

were found. In Table VI. the hind leg lengths of pituitary-thyroidless tadpoles normally fed and killed more than a month later than the above are found to be in sharp contrast to these. None of these pituitary-thyroidless *Rana pipiens* tadpoles were brought to metamorphosis by iodine feeding but there is little doubt but that it will be possible to do so; at any rate there is an extremely strong tendency in that direction.

TABLE VI.

Rana pipiens WITH PITUITARY AND THYROID GLANDS REMOVED.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Date Killed.
1.	23.7	10.2	.43 K.	Aug. 22.
2.	28.3	11.2	.53 K.	Aug. 22.
3.	49.0	19.5	.76 K.	Oct. 7.
4.	59.8	23.3	2.28 K.	Aug. 21.

TABLE VII.

SIZE OF THYROID GLANDS AND COLLOID MASSES OF PITUITARYLESS *Bufo* TADPOLES—IODINE FED. NUMBERS OF SPECIMENS CORRESPOND TO THOSE OF TABLES I. AND II.

Dimensions of Thyroid Gland.

No.	Total Length, Mm.	Body Length, Mm.	Side Length, Mm.	Width, Mm.	Thickness, Mm.	Volume, Cmm.	Aver. Vol., Cmm.	Aver. Diam. Colloid Mass, Mm.
1.	9.3	3.93	R. .100	.064	.031	.000198	.000209	.014
			L. .100	.071	.031	.000220		.014
3.	8.2	4.29	R. .137	.080	.037	.000405	.000409	.012
			L. .157	.080	.033	.000414		.014
7.	9.9	5.38	R. .149	.090	.036	.000482	.000364	
			L. 118.	,070	.030	.000247		
8.	11.3	5.28	R. .086	.070	.046	.000276	.000275	.024
			L. .078	.080	.044	.000274		.026
9.	11.6	5.20	R. .146	.090	.029	.000381	.000396	.016
			L. .089	.140	.033	.000411		.014
12.	10.3	4.82	R. .113	.070	.027	.000213	.000444	.014
			L. .157	.100	.043	.000675		.020
13.	8.2	5.15	R. .193	.100	.043	.000710	.000547	
			L. .150	.080	.032	.000384		
14.	9.3	6.34	R. .190	.121	.040	.000899	.001324	.016
			L. .220	.150	.053	.001749		.016
	9.8	5.04					.000471	.016

The iodine-fed pituitaryless *Bufo* tadpoles shown in tables I. and II. were sectioned and their thyroid glands studied, with the object of seeing whether the iodine feeding had brought about any notable increase of colloid in the thyroid gland. Two were preserved intact for demonstration purposes. Only eight out of the twelve tadpoles sectioned were found to be in a satisfactory state of preservation for histological study. This was due to the fact that the tadpoles were usually preserved after they had died. Although the aquaria were frequently examined, the summer heat often caused considerable disintegration of the thyroid gland before the specimens could be preserved. An examination of Table VII. shows the length, width and thickness of each thyroid gland. The specimens are designated the same as in Table I. From the dimensions of each gland was calculated the volume of a parallelopiped that would contain it. This of course would constitute but a rough relative approximation to the volume. Table VIII. shows similar measurements and calculations made upon the thyroid glands of normally fed pituitaryless *Bufo* tadpoles of size corresponding to these as measured at the beginning of iodine feeding. A comparison of Tables VII. and VIII. will show that the thyroid glands taken as

TABLE VIII.

SIZE OF THYROID GLANDS AND COLLOID MASSES OF PITUITARYLESS *Bufo* TADPOLES NORMAL FEEDING AS CONTROLS:

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Side Length, Mm.	Breadth, Mm.	Thickness, Mm.	Volume, Cmm.	Average Volume, Cmm.	Diameter of Colloid Masses, Mm.
1.	16.7	7.4	.92	R. .110	.117	.047	.000604	.000469	.013
				L. .130	.081	.069	.000335		
2.	13.7	6.6	.36	R. .060	.150	.076	.000684	.000523	.011
				L. .070	.089	.060	.000373		.010
3.	17.8	8.7	1.09	R. .130	.111	.057	.000822	.000791	.011
				L. .150	.118	.043	.000761		.016
4.	13.6	6.4	.40	R. .090	.110	.078	.000722	.000679	.011
				L. .090	.107	.061	.000587		.011
5.	14.0	6.7	.40	R. .070	.080	.036	.000201	.000230	.013
				L. .090	.072	.040	.000259		.013
6.	14.0	7.0	.56	R. .180	.094	.041	.000693	.000629	.014
				L. .160	.104	.034	.000565		.014
Aver	14.6	7.1						.000554	.013
7.	22.5	10.5	3.23	R. .330	.193	.057	.003330	.003382	.024
				L. .339	.153	.068	.003435		.024
8.	26.8	13.4	4.16	R. .460	.236	.057	.006187	.006770	.028
				L. .490	.224	.167	.007353		

a whole have shrunken appreciably together with the shrinkage of the body as a whole; but not proportionately as much. In the iodine-fed tadpoles the colloid masses show a slightly greater average diameter than do those of the normally fed specimens. These facts do not seem especially significant. The slight increase of the colloid masses and the actual decrease in the dimensions of the gland roughly counterbalance one another. It is to be admitted that the number of specimens is small but it is large enough to demonstrate that no striking changes in the size of the thyroid glands and of their colloid content in pituitaryless tadpoles result from iodine feeding, at least, not within the stages and time limits of the experiment. It will be interesting in future experiments to test whether feeding iodine at early stages prior to the normal time of colloid formation would cause it to appear precociously.

In a paper upon the "Development of the Thyroid Glands of *Bufo* and their Normal Relation to Metamorphosis," Allen (in press), a study was made of the relation of the growth of the thyroid gland to the growth and metamorphosis of *Bufo* tadpoles. The stages used are a trifle more advanced than those used in this experiment. It appears, however, that the thyroid glands are of about the same size in their early stages in pituitaryless tadpoles as in normal tadpoles and that the colloid begins to form at the same time in both.

SUMMARY AND CONCLUSIONS.

At the beginning of this paper, attention was called to the fact that the growth of the thyroid glands and the accumulation of colloid in them are retarded by the removal of the pituitary gland. Rogers and Larson have both shown that the removal of the thyroid gland causes an hypertrophy of the pituitary gland (Rogers, '18, anterior lobe; Larson, '19, anterior and intermediate lobes). It would be premature to offer an interpretation of these facts but they seem to justify the hypothesis that these glands are closely interrelated. The writer has for several years favored the hypothesis that the thyroid gland may, largely at least, play the rôle of a storage organ for the iodine of the body, at the same time controlling its distribution. It even seems possible that the pituitary gland might play the chief rôle in the utilization of iodine by the body. This view is in the main based upon the fact that tadpoles of *Rana* and *Bufo*, deprived of the pituitary gland, fail to metamorphose in spite of the fact that the thyroid glands remain intact and even though the body of the tadpole reaches gigantic size. The removal of the pituitary gland renders the thyroid gland as powerless to bring about metamorphosis as though the thyroid gland were itself removed.

It was shown by Swingle that metamorphosis could be produced in normal tadpoles by feeding them iodine. He likewise showed that iodine feeding would produce metamorphosis even in tadpoles deprived of the thyroid glands. Simultaneously with the above work, the writer was carrying on the experiments that form the subject of the present paper. They show conclusively that metamorphosis can be produced by iodine feeding in tadpoles deprived of the pituitary glands. More striking still is the demonstration of the fact that feeding iodine to tadpoles deprived of both thyroid and pituitary glands can carry them far in the process of metamorphosis, the attainment of which was prevented only by the death of the tadpoles.

In the light of all these facts, we are justified in drawing the following general conclusions:

With normal feeding, involving the intake of very minute quantities of iodine, the presence of both the thyroid and pituitary glands is essential to enable the animal to undergo metamorphosis. Unpublished data show quite conclusively that *Rana* and *Bufo* larvæ proceed to the same stages of limb growth in the absence of the thyroid or of the pituitary gland or of both together. The administration of large quantities of iodine will cause the tadpoles to undergo metamorphosis in the total absence of the thyroid gland or of the pituitary gland or in the simultaneous absence of both thyroid and pituitary glands. It may be found by later experimentation that iodine is not the only factor that is capable of bringing animals to maturity. Investigation along these lines is still in its infancy. Before this paper appears in print, the writer expects to have under way more extensive quantitative experiments that will determine the reactions of normal, pituitaryless, thyroidless, and pituitary-thyroidless tadpoles to these and to other factors that influence development.

LITERATURE.

Adler, L.

'14 Metamorphosestudien an Batrachierlarven. I., Extirpation endocriner Drusen. II., Extirpation der Hypophyse. Arch. f. Entwickelungsmech., Bd. 39.

Allen, B. M.

'16 Extirpation Experiments in *Rana pipiens* Larvæ. Science, November 24, 1916.

Allen, B. M.

'16 Effects of the Extirpation of the Anterior Lobe of the Hypophysis of *Rana pipiens*. BIOL. BULL., Vol. 32, March, 1917.

Allen, B. M.

'16 The Results of Thyroid Removal in the Larvæ of *Rana pipiens*. Jour. Exper. Zool., Vol. 24, p. 499, January 5, 1918.

Gudernatsch, J. F.

'12 Feeding Experiments on Tadpoles. Arch. f. Entwickelungsmech., Vol. 35, p. 457.

Gudernatsch, J. F.

'14 A Further Contribution to the Knowledge of Organs with Internal Secretion. Amer. Journ. Anat., Vol. 15, p. 431.

Larson, M. E.

'19 Effect of the Extirpation of the Thyroid Gland upon the Pituitary Gland in *Bufo*. Anat. Record, Vol. 15, p. 353.

Rogers, James B.

'18 The Effect of the Extirpation of the Thyroid upon the Thymus and the Pituitary Glands of *Rana pipiens*. Jour. Exper. Zoöl., Vol. 24, p. 589, January 5, 1918.

Smith, P. E.

'16 Experimental Ablation of the Hypophysis in the Frog Embryo. Science, August 25, 1916.

Smith, P. E.

'16 The Effect of Hypophysectomy in the Early Embryo upon the Growth and Development of the Frog. Anat. Record, October, 1916.

Swingle, W. W.

'19 Studies on the Relation of Iodine to the Thyroid. I. The Effects of Feeding Iodine to Normal and Thyroidectomized Tadpoles. Jour. Exper. Zoöl., Vol. 27, p. 397, January 5, 1919.

Swingle, W. W.

'19 Studies on the Relation of Iodin to the Thyroid. II., Comparison of the Thyroid Glands of Iodin-fed and Normal Frog Larvæ. Jour. Exper. Zoöl., Vol. 27, p. 417, January 5, 1919.

THE MIGRATION OF REPRODUCTIVE ORGANS FROM PARENT TO BUDS IN HYDRA.

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The germ cells of hydra, as far as we are able to interpret by means of a microscopic study of carefully prepared sections, originate directly from the ordinary interstitial cells. It is true, however, that some investigators advocate the specificity of the germ cells. But the species studied gave no evidence of a specific line of germ cells. It is not my intention to take up a careful study of the germ cells in hydra, as to their method of origin and history, but rather to consider them from the standpoint of their place of origin. That is, do the reproductive organs originate directly on the forming buds, or are they formed on the parent hydra and later migrate to the buds during their development.

Investigators have reported the presence of sex organs on the buds in hydra, but in no case has it been definitely stated that these organs originated directly from the ectoderm of the young buds on which they were found.

Fig. 1 represents a camera drawing of *Hydra vulgaris* in a partially contracted condition, with five buds in different stages of development, and thirteen spermaries. Most of the spermaries were completely formed before budding begun. In no instance did the spermaries originate directly on the buds, but migrated with the cells of the parent to those of the forming buds.

Diagram A, 1, represents a small portion of the parent hydra extended, with a bud in its initial stage of development. The area between *R-G* on either side of the bud, indicates the region of growth or the region that contributes directly to the formation of the bud. Reproductive organs found within this region (*R-G*), migrate from the parent hydra to the bud. Those outside of this region of growth never reach the bud, but in most instances retain their original position, while the buds are developing. Those on the border line, often migrate towards the buds but never reach them. That is, they remain on the parent. The place occupied by the reproductive organs on the completely formed bud is determined by their position in the growth region (*R-G*) of the parent hydra at the time the bud starts.

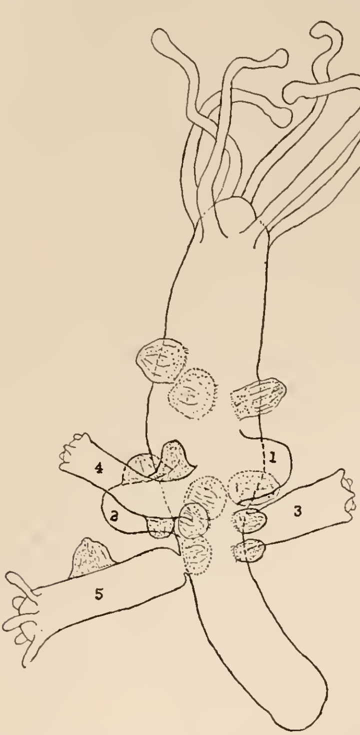
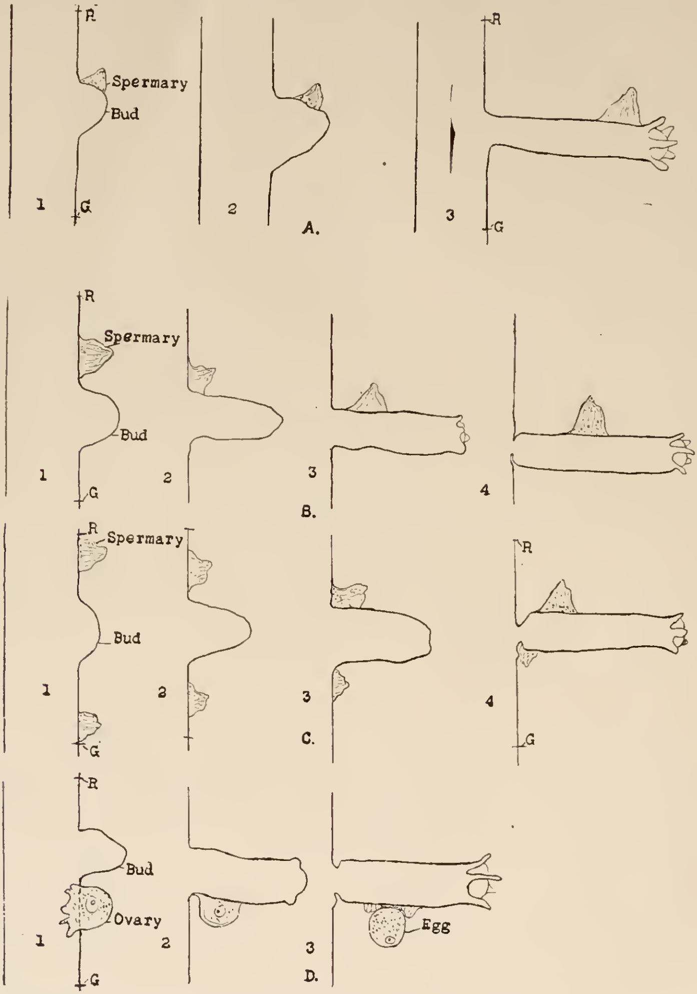


FIG. 1. Hydra with 5 buds and 13 spermaries. Spermaries have migrated from parent to buds 2, 4 and 5.

Spermaries or ovaries found near the tip or center of the distal end of the bud when it makes its first appearance, take up a final position at the distal end of the completely formed bud (Diagram A, 3). The spermary (Diagram A, 1–3) during the different stages of progress in the development of the bud, retains its same position with reference to the tip or distal end. In Diagram A the bud became independent of the parent in sixty hours after its initial stage of development. Diagram B, 1, 2, 3 and 4 shows the position of the spermary at different periods of growth on the bud, and its final position when the bud is completely formed. Note the position of the spermary before its migration began. In Diagram C, 1, 2, 3 and 4 the spermaries were located near the outer edge of the region of growth (*R-G*). The time required for the development of the


R
Spermary
Bud
1
G
2
A.
R
3
G
R
Spermary
Bud
1
G
2
3
B.
4
R
Spermary
Bud
1
G
2
3
C.
R
4
G
R
Bud
Ovary
1
G
2
Egg
3
D.

Diagrams A, B, C and D to show the migration of reproductive organs from parent hydra to the forming buds.

bud in Diagram C was seventy hours. Diagram D, 1, 2 and 3 shows the position taken by the ovary, at the different stages in the development of the bud. The rate of growth and migration of the ovary is the same as in the case of the spermary.

The position taken by the reproductive organs during the different periods of growth in the formation of the buds, indicate beyond question, that the region around the base of the buds, contributes almost entirely to the formation and growth of the buds, and that the cells of the buds contribute but little to their own development. The cells in the growth region not only divide mitotically, but migrate from the parent hydra to the buds and carry the reproductive organs with them.

It was not an unusual thing to find hydra that were produced by budding in the different cultures, which showed either ovaries or spermaries at their extreme distal or proximal end. Diagrams A, 3, and C, 4, are good examples of these conditions.

In a few instances where the spermary was situated equidistant between two forming buds, the spermary became divided through the central region, either half reformed into a distinct spermary and migrated towards the corresponding bud. In these particular cases the spermaries were in their early stages of development. But in those spermaries that were completely formed and contained mature sperm, no division occurred. They either remained in their former position equidistant between the two buds, or migrated in toto towards one or the other.

The migration of the reproductive organs as observed in hydra is rather an interesting phenomenon, and occurs quite frequently, especially in budding hydra that are rich in sex organs. This process of migration however, is directly associated with asexual reproduction and can not be considered as having any very special significance, since the reproductive organs migrate in conjunction with the surrounding cells and not independently of them.

LITERATURE CITED.

Downing, E. R.

'04 The Spermatogenesis of *Hydra*. Zool. Jahr., Band 21.

Lang, Albert

'92 Über die Knospung bei *Hydra* und einigen Hydropolypen. Z. wiss. Zool., Vol. 54.

Tannreuther, G. W.
'09 Observations on the Germ Cells of *Hydra*. Biol. Bulletin, Vol. XVI.
'09 Budding in Hydra. Biol. Bulletin, Vol. XVI.
Wagner, R. E.
The Oögenesis and Early Development of *Hydra*. Biol. Bulletin, Vol. XVII.

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RESISTANCE OF CILIA TO CYTOLYTIC AGENTS

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In studying the hæmolysis of erythrocytes from a series of mammals, Rywosch ('07) found that the order of resistance to saponin was almost the reverse of that to hypotonic NaCl. He did not however offer an explanation. Hoeber in 1912 suggested that saponin might affect the lipoids, but that the hypotonic solutions attacked some other component of the cell. K. Meyer (reviewed by Port) noted that corpuscles rich in cholesterol were also most resistant to saponin. The protective action of cholesterol in a cholesterol-lecithin mixture against saponin has been demonstrated on artificial membranes, and several workers (reviewed by Port, '10) very early cited Abderhalden's analyses of erythrocytes in support of the view that cholesterol is the chief factor in determining saponin resistance. Hoeber ('08 and '14) used several hypotonic salt solutions, and found that the phosphates were least injurious if used alone, but if mixed with saponin became the most injurious of the series. He interpreted this as due to the action of PO_4 on the cell colloids, but not until 1914 did he offer the following more elaborate theory. A cell protein which normally is stabilized by PO_4, will, after undergoing a change in sign (as by the action of acids), be destroyed by PO_4. Saponin produces such a change in sign of the corpuscles and therefore attacks especially cells rich in PO_4. This may explain the fact that a cell resistant to hypotonic NaCl is relatively susceptible to saponin.

The present experiments were undertaken to find whether this curious reversal of resistance occurs in cells of another sort (ciliated cells of various marine invertebrates), and to compare the effect of hypotonic NaCl with that of a hypotonic solution which is at the same time balanced, *e. g.*, dilute sea water.

The ciliated gill filaments of several molluscs and the ciliated larvæ of echinoderms, molluscs and annelid worms furnished material for the experiments. The gills were divided into narrow strips of one or two filaments. The larvæ were generally heliotropic and so could easily be secured in large numbers in very little water. Not all the larvæ were fully normal: *Echinarachnius*, *Arenicola* and *Asterias* were the most variable in vitality. All of the solutions used (except of course NaCl) were made up in sea water rather than in distilled water and were never kept more than eight hours: not over four hours in the case of volatile substances. The experiments were carried out in stoppered test tubes which for higher temperatures were floated in warm water. The temperature of the tubes was read during every experiment. Each day's results were recorded on a separate slip of paper. The summary given below is an average of all observations and where these vary because of temperature, difference in viability (or other experimental error), the order of susceptibility has been verified by reference to the separate experiments in which the conditions must have been the same. The results obtained are as follows:

In comparing the resistance to saponin with that to hypotonic NaCl it is clear that although there are some irregularities (which are starred), the general order is the same. This is quite unlike the sharp reversal of order obtained with erythrocytes in saponin and in hypotonic NaCl. The order of cilia resistance in

TABLE I.

Saponin (per cent. represents dilution of a stock solution of 0.5 per cent. in sea water).

	20–23° C.							
Per Cent.	.25.	.5.	1.	5.	10.	30.	50.	100.
Echinarachnius gastr.	8	5	5	2				
Arbacia 1 d.		10	4	< 4				
" 2 d.		10	8		3			
" 3 d.	20	10+	6		3			
Echinarachnius pluteus			11	4	2			
Arenicola 2 d.			15	3	4	3		
Chætopterus 1, 2 d.			22	6	5	3		
" 3 d.			15+	16	8	4		
Nereis 1 d.			40+	12	8	5	3	
" 2 d.			45	19	9	8	4	3
" 3 d.			50		8	8	6	
Asterias 1–10 d.			30+	60				80+
Cumingia 1, 2 d.			30+					30+

Hypotonic Sea Water.

		20–23° C.			
Per Ceut.		40.	35.	30.	20.
Echinarachnius plut.		30	15	5	< 2
" "	gastr.	<30	15	5	< 2
Arbacia	1 d.		20 +	$4\frac{1}{2}$	2
Arbacia	2 d.				
"	3 d.			7	2
Arenicola	2 d.	30 +		8	5
Chætopterus	1, 2, 3 d.			20	9
Nereis	2 d.	30 +	20 +	12–22	8
Asterias	1–10 d.			18	9
Cumingia				30	22

Hypotonic NaCl (stock solution).

	20–23° C.		
Per Cent	40.	30.	20.
Arbacia	11	8	
Arenicola	25 +	5	
Echinarachnius	15 +	< 2	
*Asterias 6 d.	15	18	15
**Cumingia*		25	15
Chætopterus	25	27	
Asterias 2, 7, 8 d.	7	25	10
Asterias 1 d.	15	30	15
Nereis		30	4

TABLE II.

Saponin (per cent, represents dilution of a stock solution of 0.5 per cent. in sea water).

	20° C.				
Per Cent	10.	20.	30.	50.	100.
Mytilus			8	4	2
Pecten	15	15	12	10	4
Mya	20	20	10	8	8
Quahog	25 +	20 +	15	8	8
Razor clam	50	30	30	18	17
Modiola	50 +	30	30	27	20

Hypotonic Sea-water.

	20° C.			
Per Cent	20.	15.	10.	5.
Pecten	6	4		2
Mytilus	35	16	8	3
Mya	35	15	9	4
Quahog	40	20	14	4
Razor clam	45 +	35	14	4
Modiola	45 +	35	14	4

Hypotonic NaCl.

	20° C.	
Per Cent	15.	10.
Pecten	<3	<3
Mytilus	5	<3
Mya	5 +	4
Quahog		7
Razor clam		8 +
Modiola		

hypotonic sea water is almost exactly the same as in saponin solution: there is in fact only one slight difference (*Echinarachnius plut.*) as against two in NaCl. Evidently hypotonic sea water has not quite the same action on the cilia as hypotonic NaCl, but the difference is not great. No data on the hæmolytic power of a hypotonic balanced solution are available for comparison.

The action of acids upon erythrocytes (Koeppe, cited by Rywosch) is a function of their H^+ concentration, but their action upon cilia of protozoa (unpublished experiments) and of the giant clam (Harvey) is correlated with other factors as well.

Still another evidence of the peculiarity of erythrocytes is found in temperature coefficients. They show with a rise in temperature increased susceptibility to acids and to chloroform (Rywosch) but not to saponin or to hypotonic NaCl (Kagan). As the following table indicates this is not the case with most of the ciliated cells.

TABLE III.

Saponin.

	20–23° C.					25° C.			30° C.	
Per Cent.	0.5.	1.0.	30.	50.	100.	1.0.	30.	50.	0.5.	100.
Echinarachnius pl.		11				3				
Arbacia 2 d.	10	15	3			3			8	
Chætopterus 2 d.		22	3				3			
Nereis 1 d.		40	5	3				3		
Cumingia 2 d.		30			30			50		
Asterias 2 d.		60+			80			20		
" 3 d.								25		30
" 6–10 d.								60		

Hypotonic Sea Water.

	20–23° C.			25° C.		
Per Cent.	35.	30.	20.	35.	20.	20.
Echinarachnius pl.	15	5	2	10	3	2
" g.	15	5	2	20	5	
Arbacia 3 d.		7	2	25	5	2
Nereis 2 d.	20	15	8			13
Asterias 2 d.		18	9	60	15	9
" 4 d.				30	7	
" 6–10 d.					12	10

TABLE IV.

Saponin.

	20° C.					30° C.				
Per Cent.	10.	20.	30.	50.	100.	10.	20.	30.	50.	100.
Mytilus			8	4	2		4			
Pecten	15	15	12	10	4	5	4	2	2	
Mya	20	20	10	8	8		13	3	$3\frac{1}{2}$	
Quahog	25	20	15	8	8		16	5	4	
Razor clam	50	30	30	18	17	11	8	5	$2\frac{1}{2}$	3
Modiola	50	30	30	27	20	15	12	6	$4\frac{1}{2}$	4

Hypotonic Sea Water.

	20° C.				30° C.	
Per Cent.	20.	15.	10.	5.	20.	15.
Pecten	6	4		2	3	3
Mytilus	35	16	8	3	30	10
Mya	35	15	9	4	40	13

Thus resistance of cilia to both saponin and hypotonic NaCl is lowered by increasing temperature instead of remaining constant as is the case with erythrocytes. The coefficients are higher in saponin than in hypotonic sea water and also vary considerably from one form to another. This is not the case with erythrocytes. However the action of acids upon various infusoria, as upon erythrocytes, is markedly altered by change in temperature.

A few experiments were made using chloroform, ether and acetone made up in sea water (in vols. per cent., chloroform 0.1–0.2, and 0.37 per cent.; ether 2–6 per cent., acetone 10 per cent.). The gill cilia showed the same order of resistance in these reagents as in hypotonic sea water. The larval cilia were more irregular, as the following list shows, though the order is not very different from that obtaining in saponin.

CHLOROFORM.	ETHER.	ACETONE.
Echinarachnius p.	*Echinarachnius* g. & p.	*Echinarachnius*
" g.	*Arbacia* 1, 3 d.	*Arbacia* 2, 3
Arbacia 1 d.	**Cumingia*	" 1
**Asterias* 3–4	*Asterias* 3, 6–10 d.	**Asterias* 2–6, 10
* " 2	*Chætopterus*	**Cumingia*
Arenicola 2	*Nereis* 1, 2	*Chætopterus*
Nereis 2	**Arenicola*	**Arenicola*
Cumingia	*Asterias* 1, 2	*Asterias*
Asterias 6–10		

With erythrocytes Rywosch found in each of these reagents a quite different order, more irregular than was observed in the present experiments.

An adequate explanation of these results is impossible at present. Although detailed analyses of corpuscles have long been available, there are analyses of only two of the organisms used in the present experiments, made upon the eggs (not larvæ) of *Arbacia* and *Asterias* by Matthews. He found the *Arbacia* egg rich in cholesterol as compared with lecithin and the *Asterias* egg the reverse. If these lipoids alone determined resistance to saponin and to hypotonic NaCl, *Arbacia* should be more resistant than *Asterias*, but this is not the case. This may be due to a change in the cholesterol content as the egg develops into a larva. Certainly differences due to age occur in most of the forms, especially at times of great morphological change. However, until we have adequate analyses we cannot offer a definite explanation of the characteristic differences in the behavior of these ciliated cells toward reagents.

SUMMARY.

1. Cilia which are resistant to saponin are generally resistant to hypotonic sea water and hypotonic NaCl. The reversal of resistance found with erythrocytes does not hold with cilia.

2. Relative resistance of cilia to hypotonic NaCl is not the same as resistance to hypotonic sea water, although the irregularities are not numerous.

3. Most of the cilia show a change in resistance with change in temperature. This is more marked in saponin than in hypotonic sea water.

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BIBLIOGRAPHY.

Harvey
'14 Internat. Zeit. f. Ph-Chem. Biol., I.
Hoeber
'13 and '14 Physikalische Chemie d. Zelle, 2te u. 3te Aufl.
'08 Biochem. Zeit., 14.
'14 Biochem. Zeit., 60, p. 131.
Kanitz
Winterstein's Handb. d. vergleich. Physiol. (for analyses of corpuscles).
Kagan
'13 Folia Hæmatologica, 17, p. 211.
Matthews
'13 J. Biol. Chem., 14, p. 465.
Port
'10 Deutsch. Arch. klin. Med., 99, p. 259.
Rywosch
'07 Pflueger's Archiv, 116, p. 229.

WH 17K8 %